Living kidney donation

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Living kidney donation:
implications for donor screening and follow-up

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General introduction and aims of the thesis

Transplantation

Worldwide, the incidence and prevalence of chronic kidney disease is rising. When chronic kidney disease evolves to end stage kidney failure, renal replacement therapy becomes necessary. Although dialysis can replace kidney function, a new, transplanted kidney can restore renal capacity in a continuous and more effective way. The first successful kidney transplantation was performed in Boston 1954, by Murray and colleagues (1). A young man with chronic kidney disease received a kidney from his identical twin brother. The graft and recipient survived for eight years; Murray received the Nobel Prize for Medicine in 1990. Since then, many medical breakthroughs have led to improved outcomes of kidney transplantation. The development of immunosuppressive drugs solved part of the problems of rejection of the graft, and enabled transplantation between genetically non-identical subjects. This also paved the way for using organs from post-mortem donors for transplantation. Transplantation has now become the preferred treatment for end stage kidney disease, with better quality of life and longer survival when compared with patients on dialysis (2-6). Despite all this, donor shortage is a still a major and persistent problem, and optimization of the donor pool is of cardinal importance in transplantation medicine. The possibility of living kidney donation, which is in line with the first transplantation by Murray, provides a potentially huge donor pool. However, a proper balance between optimal enlargement of the donor pool and long-term donor safety must be ensured to justify the living kidney donor program.

Living kidney donation

A single, healthy kidney has sufficient capacity to cover the entire workload of two kidneys. Nevertheless, the single kidney state literally puts a lot of pressure on the remnant kidney, making good health an important requirement for kidney donation. The fact that a donor kidney can be retrieved from a healthy subject without detrimental effect allows the practice of kidney donation during life. Over the last decades, living kidney donors have become more important for kidney transplantation programs worldwide (7;8), although there are large international differences in the use of living donors. Nowadays, about half of the Dutch kidney transplantations are performed with the use of living donors (9), while for example in the United States living donors account for about one third of the transplantations (10). Kidneys retrieved from living donors provide better function and longer half life after transplantation than kidneys from post-mortem donors (11-14). In the past, living kidney donors were very strictly selected, and accordingly represented the healthiest subjects of a population. This is apparent from an iconic study entitled ”Kidney donors live longer”, by Dr Fehrman-Ekholm, herself a kidney donor, showing that survival in kidney donors is better than in the general population, obviously due to selection bias (15). Many other studies confirmed that living donor outcome is good (16-24).

Under pressure of the ever growing waiting list for transplantation organs, the selection criteria for potential donors have become more liberal (25;26). The current donor pool is older, more overweight and has higher blood pressure, sometimes even in the hypertensive range. Figure 1 displays trends in donor characteristics in time for the University Medical Center Groningen showing a trend towards older, more overweight and obese donors, with since 2002, hypertension in a few cases. Since these characteristics are known risk factors for

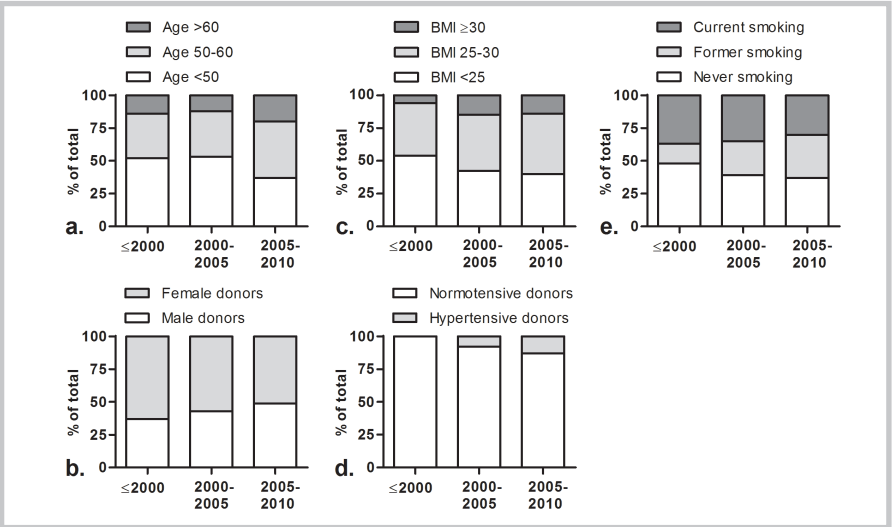

Figure 1: trends in donor characteristics in the University Medical Center Groningen over time. Bars represent percentage of donors in the subsequent time periods.

the development of kidney disease, this raises a new set of issues, and warrants increased attention for living donor safety in the current era. In addition, a trend to more male donors is seen, which may be explained by the higher prevalence of kidney diseases among women nowadays.

Effect of donor risk factors on the kidney

Abovementioned changes in the donor pool may affect the outcome of the donor as well as the recipient. Whereas in the past living donors were in excellent health, nowadays donors may be more representative of the population they are derived from, including a more prominent risk profile. This emphasizes the importance of thorough screening and accurate follow-up. Subjects at risk for kidney function loss after donation should be identified in early stages to have optimal effect of preventative strategies. Donor renal risk profile may also have consequences in a different context,

i.e. the recipient kidney function. Hereafter, the potential effects of donor characteristic s and risk factors on the kidney are discussed.

Age

As the Dutch population is getting older, the mean age of potential donors is rising as well (figure 1). With increasing age the prevalence of co-morbidities such as hypertension increases as well (27-29), therefore the sole effect of ageing is hard to study. With increasing age, kidney function declines, albeit with large individual variation. Rate estimates vary around 0.75 mL/min per year (30-33). Co-morbidities like hypertension or arteriosclerosis may accelerate kidney function loss (34). The decline in kidney function is attributed usually to nephron loss, with obliteration of the glomerular capillary bed leading to glomerular and ensuing tubular

obliteration (35;36). Remnant glomeruli may increase in size, which is associated with subsequent development of glomerular sclerosis (37-40). The tubulo-interstitium and the kidney vasculature are affected by ageing as well (41-43). Changes include for instance tubular atrophy, a decrease in the number, volume and length of tubuli, and intima fibrosis and simplification of the kidney vasculature. Even without morphological changes, the ageing kidney is more vulnerable for kidney injury, especially when induced by ischemia. Although age related changes occur with a large individual variance in presence and severity, the abovementioned changes make a kidney more vulnerable for damage with decreased adaptive capacity, which may be relevant for the remaining kidney in the donor, as well as to the transplanted kidney.

Gender

Several studies in deceased kidney donors suggested that female kidneys perform worse after kidney transplantation compared to male kidneys, especially when transplanted in a male recipient (44-47). There are several gender related differences in renal characteristics of potential relevance to kidney donation. In absolute numbers, females have lower kidney function than male subjects. This is in part due to the simple fact that in general females are smaller, and, hence, have smaller kidneys and a lower nephron number (48). This is in line with a lower metabolic demand in females, due to a smaller body size and lower muscle mass. Although in absolute mL/min kidney function may be lower, kidney function between males and females appears to be more or less similar when kidney function is scaled to body size. Furthermore, when kidney function is scaled to the extracellular fluid volume, women have similar (49) or even higher kidney function than men (50). Although women may have lower absolute kidney function, men are more prone to the development of hypertension and kidney disease (51-54). This appears to be, at least in part, hormonal, since post-menopausal women show a similar of even higher rate of progression of kidney disease as male subjects (55). Normal, age related kidney function decline, however, is higher is men (56), though there does not seem to be a gender difference in the occurrence of age related glomerulosclerosis (57).

Body mass index

The increasing prevalence of overweight and obesity in the general population has also affected the potential donor population (figure 1). Overweight, and especially obesity, are known risk factors for the development of kidney disease and even end stage renal disease in the general population (51;58-63) as well as in renal transplantation (64;65). Excess body weight is associated with the development of hypertension, diabetes mellitus and cardiovascular disease, which in their turn may be harmful to the kidney. In addition to this indirect effects of overweight on kidney disease, overweight has independent, direct detrimental effects as well. Potential mechanisms lie in activation of the renin angiotensin aldosterone system, with a subsequent increase in angiotensin II, which may be harmful to the kidney; overweight induced glomerular hyperfiltration with elevated filtration rate and filtration fraction (64), which was an independent predictor of graft loss in transplant recipient (66); and the production of pro-inflammatory cytokines by visceral fat tissue. Biopsy studies

showed glomerulomegaly, enlarged glomeruli, and glomerulosclerosis (67) in obese subjects (68-72), which may in turn lead to the development of proteinuria (71;73). Fortunately, overweight is a modifiable risk factor, and, therefore, deserves extra attention in screening and follow-up.

Blood pressure and hypertension

High blood pressure is a known risk factor for the development of kidney damage and kidney function loss (74-78). Hypertension coincides with other risk factors such as older age and obesity, and with many kidney diseases. It may accelerate kidney function decline in subjects with kidney disease (79), and is highly present in subjects with end stage renal disease (80). Thus, adequate blood pressure control is of major importance for donor and recipient. High blood pressure, necessitating the use of antihypertensive drugs, used to be an absolute contraindication for living kidney donation. As hypertension, especially of the mild to moderate category, is a frequent condition (42-51 % in the adult Dutch population) this considerably limits the pool of potential donors. Since 2002, however, hypertensive donors are allowed to donate, provided that there is reasonable blood pressure control on a maximum of two antihypertensive drugs. Up to 2010, 39 pre-existent hypertensive donors were found eligible to donate. Since long term follow-up is not available yet, it is important to meticulously monitor outcome of kidney donation by hypertensive donors, both for donors, in terms of safety, and recipient in terms of graft performance.

Smoking behavior

Smoking behavior is so far not a selection criterion for potential donors. Although donors may be advised to stop smoking, there is no special attention for smoking behavior in screening or follow-up. Smoking is, however, an important risk factor for the development of cardiovascular disease and several forms of cancer. Furthermore, smoking is recognized as a potential risk factor for the development of CKD as well. Smoking may accelerate the aging-related decline in glomerular filtration rate (81), and increase albuminuria in subjects with hypertension (82-84), in a dose-dependent manner (63;85;86). Autopsy studies suggested that smoking may affect the kidney vasculature, causing mild kidney damage. Previously, it was found that such small lesions may make the kidney more prone to nephrotoxic effects of calcineurin inhibiting drugs (87), which are invariably used post-transplantation. Of note, the effects of smoking on the renal susceptibility to damage appear to persist after cessation of smoking, as shown by a considerably increased susceptibility to renal damage in formerly smoking lung transplant recipients (88). Furthermore, smoking of the recipient prior to kidney transplantation increases the risk for graft loss (89) and mortality (89;90).

Donor screening and post-donation follow-up

The changes of the donor pool and the acceptance of donors with potential risk factors for kidney function loss emphasizes the importance of thorough donor screening. Extending of the criteria has made decision making even harder. Where in the past donors with any risk factor were rejected, nowadays, the actual risk for post-donation kidney damage in a donor with potential risk factors has to be considered carefully. The feasibility of a living donor

program is dependent on guaranteed donor safety on long term. This emphasizes the importance of regular donor follow-up as well. If any problems do occur post-donation, early detection can prevent further damage and kidney function loss. There are, however, no strict guidelines for donor screening and follow-up, and programs differ substantially between centers. As to screening, centers differ in cutoffs of donor characteristics. Where some guidelines suggest an absolute cutoff of 80 mL/min/1.73m^2 for kidney function, others use age adjusted cutoffs. Upper limits for age can be amplified by considering older donors (>70 years) only for older recipients (>60 years). BMI is mainly associated with surgical complications, and is, therefore, not a strict selection criterion in all guidelines. Although hypertension is no longer an absolute contraindication for kidney donation, definitions differ between centers.

For follow-up, the guidelines suggest 12 to 24 months active follow-up by the transplantation center, and then life-long follow-up by local health care providers. Follow-up should focus on kidney function, development of hypertension, diabetes and obesity. In addition, most guidelines suggest psychosocial follow-up as well. Donors with a specific disease history, for example stone disease, deserve special attention to avoid complications on the long term.

Suitability of estimated kidney function for monitoring post-donation kidney function

Monitoring of kidney function is an important part of living donor follow-up. Gold standard kidney function measurement, however, is laborious and expensive. Kidney function equations estimate kidney function from a single serum creatinine sample, and are, thus, cheaper and easier in use. The two most widely used equations, the Modification in Diet and Renal Disease (MDRD) Study equation (91) and the Cockcroft-Gault equation (92), were both derived from populations with reduced kidney function. This has resulted in a poor performance in subjects with a kidney function > 60 ml/min, with considerable systematic underestimation and low accuracy, in particular in subjects without renal function impairment (93-100). A new equation was developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), which was developed in a large data set in different populations, including subjects without renal disease (101). It performs better in normal and higher ranges of GFR (102).

Donor nephrectomy elicits a major drop in kidney function. Thus, although prior to donation kidney function is normal, post-donation kidney donation becomes in range where the estimating equations have their best performance. Therefore, the equations might be suitable for use in longitudinal follow-up after donation. The validation of the equations, however, so far mainly relies on cross-sectional data. Longitudinal studies are sparse, and were mainly conducted in transplant recipients (103-108) and diabetic patients (109;110). In non-diabetic CKD patients no studies are available with a follow-up beyond 4 years (111-113).

Use of biopsies for donor screening

To improve the process of donor screening, kidney biopsies can be performed prior to donation, but for obvious reasons this has not become part of donor screening programs. However, taking a kidney biopsy during the donation procedure can provide important

information on renal morphology, without added invasiveness. Due to the large functional reserve capacity of the kidney, minor morphological and histological damage can be present without other signs of damage, such as proteinuria or decreased kidney function. Several studies in potential donors showed that minor structural damage is present among healthy subjects (22;40;114;115), although the consequences for donor outcome are unclear. Where one study reported no relation between morphological kidney damage and the development of kidney disease in the donor (116), many studies found associations between baseline damage and graft performance in the recipient (117-125). Thus, kidney biopsies may potentially provide an important source of additional information on the donor kidney, that may contribute to the assessment of renal risk for the donor and the recipient.

Aims of the thesis

The aim of this thesis is to evaluate the effects of the changing donor risk profile pool on living donor and recipient outcome. Part one of the thesis evaluates the feasibility of the use of kidney function equations for screening and follow-up of living kidney donors. Part two focuses on long term post-donation outcome of the living donor, on the effects of the current donor risk profile, and evaluates the value of time-of-donation biopsies in predicting donor outcome. Part three extends the influence of the donor to recipient outcome.

Outline of thesis

Part one: estimated GFR in screening and follow-up

Kidney function equations are known to perform poorly in subjects with normal kidney function. It is unknown whether this poor performance is due to differences between subjects with normal and impaired kidney function, or to the level of kidney function as such. Since kidney donors have a major decrease in kidney function over donation, the latter may make estimating equations more suitable in the post-donation follow-up. Furthermore, this setting will allow to establish whether the performance of kidney function equations is related to kidney function as such, as the other individual characteristics remain the same before and after donation. In **chapter one** we compare the performance of three estimating equations in an within individual analysis in kidney donors prior to and post-donation. Furthermore, longitudinal use of the estimating equations has not been evaluated for long term follow-up. To evaluate long term kidney function monitoring with kidney function equations, we compared the use of these equations to gold standard kidney function measurement in long term follow-up of CKD patients in **chapter two**. To further evaluate the use of estimated kidney function in the follow-up of living kidney donors, in **chapter three** we compare the measured and estimated course in kidney function in the post-donation setting in living kidney donors.

Part two: Effect of donor risk profile on donor renal outcome

Although previous studies showed excellent living donor outcome, the current donor pool may be at increased risk for kidney function loss post-donation. Part two of this thesis evaluates the effect of several donor characteristics on donor outcome. Since 2002, donors with well regulated hypertension are accepted for donation at the UMCG. In **chapter four** we

compare the short term and preliminary one and five year outcome of pre-existent hypertensive donors to that of matched control donors. To look in a broader perspective, **chapter five** evaluates the effects of the donor risk profile, i.e. older age, higher BMI and smoking behaviour, on five year donor outcome, and establishes a model to predict post-donation outcome. Since donors have lowered kidney function post-donation, many regard former kidney donors as having CKD post-donation merely based on the level of kidney function, which may have severe consequences on social level and welfare of the donor. **Chapter six** compares post-donation course of former kidney donors to matched CKD patients to evaluate whether the predicate is justified. **Chapter seven** evaluates the presence of pro-fibrotic and inflammatory changes in kidney biopsies performed during the donation procedure, and evaluates associations with donor characteristics and the influence of these changes on short term donor outcome.

Part three: Effect of donor risk profile on recipient renal outcome
Since the altered donor pool may not only affect the donor but also the recipient, part three of this thesis evaluates the effect of donor characteristics on recipient outcome. Female kidneys are often thought to be of inferior quality for use in kidney transplantation. Others, however, believe that the donor kidney adapts to the body size and metabolic demands of the recipient. In **chapter eight** we assess the influence of donor and recipient gender and the match in donor/recipient body size on early and five year recipient outcome. **Chapter nine** again looks at the effect of the total donor risk profile, but here we focus on the one and five year outcome of the recipient.

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Part one:
Estimated GFR in screening and follow-up

Chapter 1

1

Renal function equations before and after living kidney donation: a within-individual comparison of performance at different levels of renal function

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Abstract

Background: MDRD study equation and Cockcroft-Gault (CG) equation perform poorly in the (near-) normal range of GFR. Whether this is due to the level of GFR as such, or to differences in individual characteristics between healthy subjects and CKD patient is unknown.

Methods: We evaluated performance of MDRD, CG and CKD-EPI compared to measured GFR (mGFR; ^{125}I-iothalamate) at four months prior and two months following donation in 253 consecutive living kidney donors (mean age 50±11 years, mean BMI 26±4).

Results: mGFR declined from 103±15 to 66±11 mL/min/1.73m^2 post-donation. All equations underestimated mGFR at both time points ($p<0.001$). Arithmetic performance analysis showed improved performance post-donation of all equations ($p<0.05$), with significant reduction of bias post-donation, with median (IQR) values of 22 (20) vs 15(12) for MDRD, 14(18) *vs* 11(12) for CKD-EPI and 10(21) vs 4(14) mL/min/1.73m^2 for CG/BSA. Expressed as percentage difference mGFR-eGFR bias was reduced post-donation only for CG/BSA. Finally, in 295 unselected subjects screened for donation, mGFR was below the cut-off for donation of 80 mL/min/1.73m^2 in 19 subjects, but in 166, 98 and 74 for MDRD, CKD-EPI, and CG/BSA.

Conclusion: A higher level of GFR as such is associated with larger absolute underestimation of true GFR by eGFR. For donor screening purposes eGFR should be interpreted with great caution, and in doubt, true GFR should be performed, in order to prevent unjustified decline of prospective kidney donors.

Introduction

Nowadays, many centres rely on estimating equations to provide estimated GFR (eGFR) for renal function assessment. Many laboratories automatically report eGFR when serum creatinine is requested. The limitations, however, are well-established. In healthy subjects and subjects with renal disease with (near-) normal renal function, performance is notoriously poor, with considerable systematic underestimation of true GFR and low accuracy (1-8).

The two most widely used equations, the Modification of Diet in Renal Disease (MDRD) study equation and the Cockcroft-Gault (CG) equation, were both derived from populations with reduced GFR (9-11). Recently, a new equation was reported by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), which was developed in a large data set in different populations, including renal patients as well as healthy subjects (12). It specifically pursues better performance in normal and higher ranges of GFR.

Poor performance of renal function equations in subjects without renal function impairment has been subject of debate. Of note, the relationship between measured GFR (mGFR) and eGFR is different between healthy subjects and chronic kidney disease (CKD) patients (2,5,7,13). Differences in body composition, in particular muscle mass, and creatinine handling between healthy people and CKD patients were suggested to determine performance (2,14). Alternatively, level of mGFR as such was shown to influence performance of eGFR (7,13,15-17). However, these were cross-sectional observations, and level of mGFR may reflect between individual differences in body composition. In longitudinal analysis of CKD patients, Lee et al showed a strong negative association between bias of eGFR and mGFR (18). Yet, these studies cannot dissect the effects of CKD – with possible impact on body composition – from those of differences in mGFR itself.

In healthy kidney donors, removal of one kidney leads to a subsequent reduction in GFR without disease associated changes in body composition. Therefore, our living kidney donor program could provide a unique opportunity to compare the pre- and early post-donation performance of eGFR in high and lower ranges of GFR in the same individuals within a limited time frame. In the current study, we evaluated whether performance of eGFR in healthy subjects is dependent on the level of mGFR.

Methods

We evaluated 253 consecutive living kidney donors who donated kidneys between 1996 and 2007 in the University Medical Center Groningen. mGFR was measured as described below four months prior to and two months after kidney donation as part of the screening program and post-donation evaluation (19). eGFR was calculated from creatinine samples drawn at these same days. Procedures were conducted in accordance with the Helsinki declaration.

For evaluation of practical consequences for donor screening, data from 295 subsequent subjects screened for donation, without selection criteria other than referral for donation were analysed: the abovementioned 253 subjects and 42 subjects who all completed the full screening program, but did not donate for a variety of medical and non-medical reasons.

GFR measurement

mGFR was measured by constant low-dose infusion of the radio-labelled tracer ^{125}I iothalamate as described by Apperloo et al (20). Simultaneously, effective renal plasma flow was measured as the clearance of ^{131}I-hippurate. For the measurements, subjects were seated in a quiet room in, in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer and P represents the tracer value in plasma at the end of each clearance period. mGFR was calculated from UV/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for mGFR is 2.5%.

Calculations

The four variable MDRD Study equation was used, which is standard for creatinine samples traceable to the Isotope Dilution Mass Spectrometry (IDMS). MDRD Study, and CG equations were calculated as follows (serum creatinine (SCr) in mg/dL and bodyweight in kg). (9).

MDRD = $175 * (SCr)^{-1.154} * (age)^{0.203}$ (* 0.742 if female).

Cockcroft-Gault = (140 – age) * body weight/(72 * SCr) (* 0.85 if female).

For CKD-EPI equation the following calculations were used (12):

Female with SCr ≤0.7: GFR = $144 * (0.993)^{age} * (SCr / 0.7)^{-0.329}$

Female with SCr > 0.7: GFR = $144 * (0.993)^{age} * (SCr / 0.7)^{-1.209}$

Male with SCr ≤ 0.9: GFR = $141 * (0.993)^{age} * (SCr / 0.9)^{-0.4111}$

Male with SCr > 0.9: GFR = $141 * (0.993)^{age} * (SCr / 0.9)^{-1.209}$

Both the MDRD Study and CKD-EPI equations, no correction for ethnicity was applied as none of the donors were African Americans. BSA was calculated as according to DuBois (21). mGFR and CG were normalized by dividing the raw sample by BSA and multiplying it with 1.73, giving mGFR/BSA and CG/BSA. Mean arterial pressure (MAP) was calculated as: MAP = ((2 * diastolic pressure) + systolic pressure)/3.

Analysis of predictive performance

Performance of MDRD study, CKD-EPI and CG/BSA against mGFR/BSA was analyzed as proposed by Bostom (22) and Stevens (23), presenting bias, precision, and accuracy. Bias was calculated as median of the absolute difference (mGFR - eGFR) and of the percentage difference ((mGFR – eGFR) / mGFR * 100), giving a numeric or arithmetic value and a relative value. Precision represents the overall 'fit' of the new model against the gold standard. It is

represented by the interquartile range (IQR) of (mGFR- eGFR), and the R^2 of the linear regression of eGFR on mGFR. Accuracy reflects the proportion of subjects with eGFR values within +/- 30% of mGFR (P_{30}).

Calibration of creatinine samples

In our centre, from blood samples drawn after 1st March 2006, serum creatinine was measured by enzymatic assay on the Roche Modular. Before this date, samples had been measured by Jaffé alkaline picrate assay, on the MEGA, Merck KGaA, Darmstadt, Germany. Both methods were calibrated to the reference standard, i.e. Cleveland Clinic Laboratory measurements, as proposed by Coresh et al (24). To this purpose, a total of 516 blood samples with a broad range of creatinine were sent to the Cleveland Laboratory, of which 177 were from before March 1, 2006. Samples for calibration purposes were stored at -80°C until measured on the Roche <P> module Enzymatic assay with verified traceability to the reference standard IDMS. Calibration equations were as follows: calibrated serum creatinine = [-0.300 + 1.217 * (UMCG Jaffé creatinine values in mg/dL)] for measurements before 1st March and [0.011 + 1.087 * (UMCG Roche creatinine values in mg/dL)] for measurements after 1st March.

Statistical analysis

Analyses were performed using SPSS software version 16.0 and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [IQR]. Paired Sample's t Test and Wilcoxon Signed Ranks test were used to analyse differences between pre- and early post-donation values. To compare accuracy at both time points, Chi^2 test was used. For all three equations, eGFR was plotted on true mGFR/BSA and linear regression was performed to display differences in the relationship from pre- to post-donation. To limit influence of extreme outliers, 95% of the data was used for regression analysis. Furthermore, pre- and post-donation biases were stratified according to tertiles of pre donation mGFR/BSA. Univariate and backward multivariate analysis were performed to evaluate determinants of pre- and post-donation bias.

To evaluate the value of eGFR for donorscreening, Bayes' theorem was applied to predict probability of an mGFR<80 given an eGFR<80 mL/min/1.73m^2. Calculations were as follows: posterior change = (prior * likelihood) / evidence, i.e. $p(A \mid B) = [p(A) * p(B \mid A)] / p(B)$. For the analysis a break-up by gender, median age (50) and normal weight or overweight was made.

Results

Donor characteristics before and early after donation are given in Table 1. Estimates from MDRD Study, CKD-EPI and CG/BSA equations all significantly underestimated mGFR/BSA, at both time points (all p< 0.001). Over donation, blood pressure (MAP) increased slightly but significantly (p<0.001), BMI was unchanged. In a subset of 91 donors, paired measurements of 24 hour urinary creatinine excretion were available. Pre-donation 24 hour creatinine excretion was similar to post-donation values, 1.60 ± 0.53 and 1.61 ± 0.67 g/day respectively,

consistent with stable muscle mass as a partial index of body composition at both time-points.

After donation, all donors had lower kidney function and higher serum creatinine, reflecting loss of one kidney (table 2). The absolute mGFR decline as well as the percentage decrease after donation was significantly underestimated by each of the tested equations (all p<0.02), although the percentage decrease of MDRD and CKD-EPI were virtually constant to the change in mGFR.

Performance of renal function equations

Performance of MDRD study, CKD-EPI and CG/BSA before and after donation is given in Table 3. For the arithmetic values, performance was better post-donation (p<0.01). This is consistent with the higher R^2 of regression of eGFR on mGFR post-donation. The relative difference

Table 1: Donor characteristics before and short term after donation. p-Values in table represent post-donation values compared to pre-donation values

	Pre-donation		Post-donation		*P* Value
	Mean ± SD	Median [IQR]	Mean ± SD	Median [IQR]	
% Female	57	-	57	-	-
Age (years)	49.5 ± 10.5	49.8 [13.0]	50.0 ± 10.5	50.5 [12.7]	By default
MAP (mmHg)	92± 9	92 [11]	93 ± 9	94 [14]	0.008/0.024
Body mass index ($kg*m^{-2}$)	26 ± 4	26 [5]	26 ± 4	26 [5]	NS
Serum creatinine (mg/dL)	0.88 ± 0.16	0.87 [0.23]	1.32 ± 0.26	1.30 [0.35]	p<0.001
mGFR (mL/min)	115 ± 20	114 [27]	73 ± 13	72 [16]	p<0.001
mGFR/BSA (mL/min/1.73m²)	103 ± 15	102 [22]	66 ± 11	66 [14]	p<0.001
Estimated GFR(mL/min/1.73m²)					
MDRD Study	81 ± 15[a]	78 [18][a]	51 ± 9[b]	49 [12][b]	p<0.001
CKD-EPI	89 ± 14[a]	88 [22][a]	56 ± 11[b]	54 [15][b]	p<0.001
CG/BSA	95 ± 20[a]	94 [26][a]	63 ± 13[b]	62 [16][b]	p<0.001

Data in mean ± SD or median [inter quartile range]. MAP: mean arterial pressure; mGFR: measured GFR. p<0.001 compared to [a] pre-donation and [b] post-donation mGFR/BSA.

Table 2: Change in mGFR and eGFR before and after donation

	mL/min/1.73m²		% of pre-donation (e)GFR	
	Mean ± SD	Median [IQR]	Mean ± SD	Median [IQR]
Change in mGFR/BSA	37 ± 10	37 [12]	36 ± 7	36 [9]
Change in MDRD	31 ± 11*	29 [13]*	37 ± 9*	37 [11]
Change in CKD-EPI	33 ± 10*	33 [13]*	37 ± 9*	38 [10]*
Change in CG/BSA	32 ± 12*	31 [14]*	34 ± 8*	34 [10]*

Data in mean ± SD or median [inter quartile range]. mGFR: measured GFR. * p<0.001 compared to change in mGFR/BSA.

however suggests similar performance of MDRD Study and slightly worse performance post-donation for CKD-EPI with a larger median difference and IQR ($p<0.05$). Overall accuracy, as estimated by the P_{30} remains stable for MDRD Study and CKD-EPI equations, whilst it increases for CG/BSA ($p<0.001$ by Chi square). The individual values of the change in bias over donation are given in figure 1, for bias in mL/min (left panel), and for bias as a percentage change (right panel).

Correlation of delta relative bias and the amount of decrease of mGFR over donation showed positive relations for all equations, i.e. a larger drop in mGFR correlates to a larger decrease of relative bias. R^2 for the drop in mGFR in mL/min were 0.22, 0.30 and 0.31, and for the change in mGFR as percentage of pre-donation mGFR 0.23, 0.25 and 0.33 for MDRD, CKD-EPI and CG/BSA respectively (all $p<0.01$).

Table 3: Pre- and post-donation performance for MDRD, CKD-EPI and CG/BSA.

	Difference between mGFR and eGFR (mL/min/1.73m²)		Percentage difference between mGFR and eGFR			
	Median	IQR	Median	IQR	R^2	P_{30} (%)
Pre-donation						
MDRD Study	22 (20-25)	20 (14-26)	22 (20-24)	17 (12-21)	0.16	73 (68-79)
CKD-EPI	14 (11-16)	18 (14-22)	13 (11-16)	16 (13-21)	0.20	89 (85-93)
CG/BSA	10 (7-12)	21 (16-29)	10 (7-12)	21 (16-28)	0.14	90 (86-94)
Post-donation						
MDRD Study	15 (14-16)*	12 (9-15)	23 (21-25)*	17 (12-21)	0.31	71 (65-76)
CKD-EPI	11 (9-11)*	12 (10-16)	16 (14-19)*	20 (15-23)	0.35	89 (85-93)
CG/BSA	4 (2-5)*	14 (11-18)	6 (3-9)*	22 (11-27)	0.31	93 (90-96)

Data in median (95% CI) and inter quartile range (95% CI). mGFR: measured GFR. * $p<0.05$ compared to pre-donation value.

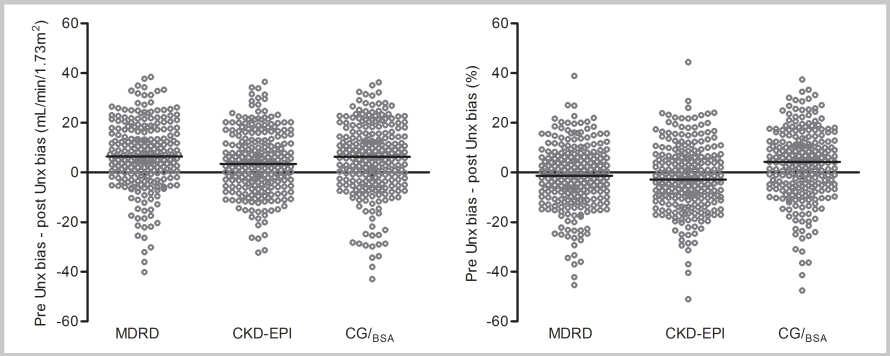

Figure 1. Individual values of the change (delta) in bias over donation (Unx). Delta was calculated as [pre-donation bias – post-donation bias] in mL/min/1.73m² for arithmetic values (left panel) and as a percentage difference for relative values (right panel). Error bars represent median bias.

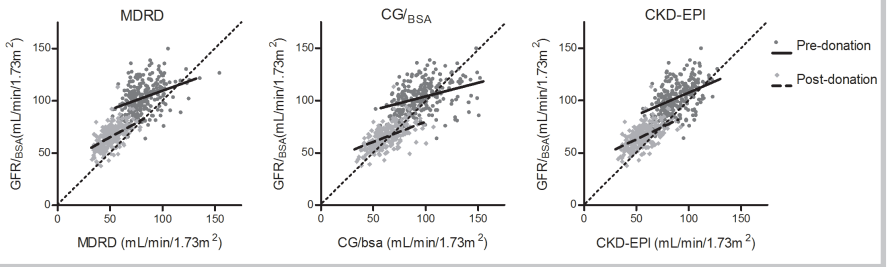

Figure 2. Regression of eGFR on mGFR/BSA for all three equations for pre- (dark dots; solid line) and post donation (grey diamonds; dotted line) values. Pre- and post-donation slopes of the regression lines are respectively 0.36 ± 0.05 and 0.55 ± 0.05 for MDRD study, 0.43 ± 0.06 and 0.47 ± 0.04 for CKD-EPI and 0.26 ± 0.04 and 0.39 ± 0.04 for CG/BSA equation.

An overlay scatter plot for both time points was made for all three equations (figure 2), showing the regression of GFR/BSA and eGFR before and after donation in the same plot. It shows a steeper regression slope after donation for all three equations.

Influence of level of mGFR on eGFR bias

Pre- and post-donation bias was divided according to tertiles of pre-donation mGFR/BSA. Figure 3 shows median bias for pre- and post donation arithmetic and relative values for each equation. Pre- and post donation arithmetic bias was higher in the higher tertiles of mGFR for all equations. For relative values, post-donation CKD-EPI and CG/BSA showed stable bias over the tertiles.

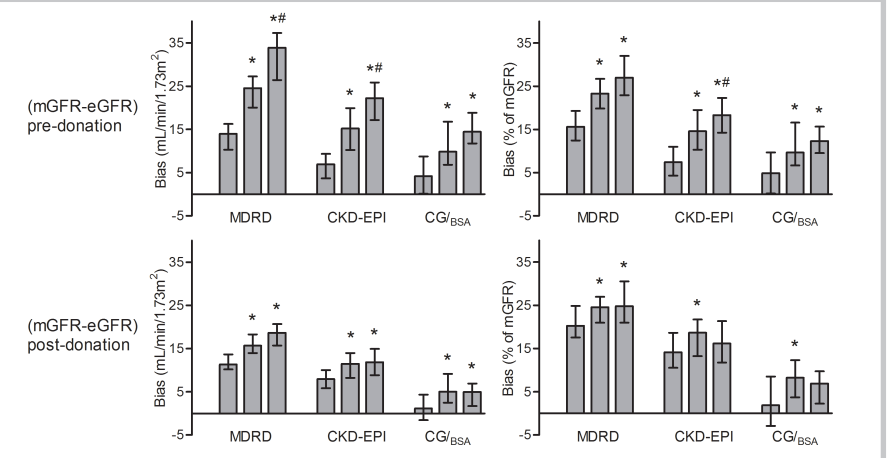

Figure 3. Pre- and post-donation bias as arithmetic (left) and relative values. Arithmetic bias was calculated as [mGFR – eGFR], relative bias as [(mGFR – eGFR) / mGFR * 100]. Bias was divided according to tertiles of pre-donation mGFR/BSA, thus pre- and post-donation tertiles contain the same donors. Tertile median [IQR] values of pre donation mGFR/BSA were 87 [82-92], 102 [99-106] and 118 [113-125] respectively. Corresponding values for post donation mGFR/BSA were 56 [53-62], 66 [61-71] and 73 [68-77] mL/min/1.73m^2. Bars represent median values with 95% confidence intervals. * p< 0.05 compared to first tertile, # p<0.05 compared to second tertile.

Table 4: Univariate analysis of pre- and post-donation bias

	Age (R^2; p)	BMI (R^2; p)	Gender (R^2; p)
Pre-donation			
MDRD Study	0.012; 0.04	NS	NS
CKD-EPI	NS	NS	NS
CG/BSA	0.067; <0.001	0.067; <0.001	0.024; <0.01
Post-donation			
MDRD Study	0.017; 0.02	0.011; 0.05	NS
CKD-EPI	NS	0.017; 0.02	NS
CG/BSA	0.102; <0.001	0.127; <0.001	0.026; <0.01

Data represent R^2 and *P*-value from linear regression. BMI: body mass index.

Anthropometric determinants of bias before and after donation

Univariate analysis of determinants of pre- and post-donation bias is listed in table 4. Multiple significant factors on univariate analysis were combined in backward multivariate analysis. Bias in the MDRD Study equation post-donation was best predicted by a model with age and BMI (R^2 0.03; p<0.01). Pre- and post-donation bias of CG/BSA was predicted by all tested factors: age, BMI and gender (R^2 0.28 and 0.26 respectively, both p<0.001). Bias of CKD-EPI was not significantly influenced by these factors.

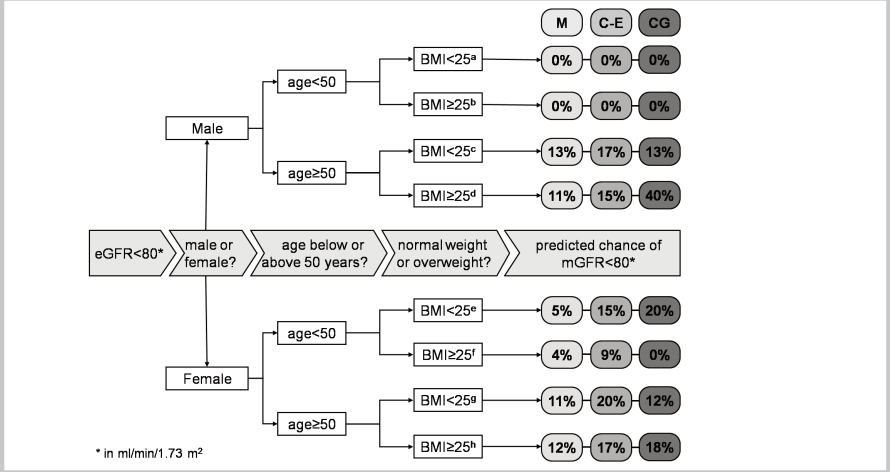

Figure 4. Predicted probability of mGFR < 80 when eGFR <80 mL/min/1.73m^2 by Bayes' theorem. Percentages represent probabilities calculated by Bayes' theorem, for MDRD (M) study, CKD-EPI (C-E) and CG/BSA (CG) equation respectively in 295 subsequent subjects referred for donor screening without prior selection. Break-ups are made for gender, age and BMI. The number of subjects in each subgroup is as follows: a n=20, b n=41, c n=25, d n=45, e n=39, f n=43, g n=34 and h n=48.

Practical consequences for donor selection by a Bayesian approach

Finally, to address practical consequences for donor selection, we calculated the probability that a potential donor with an eGFR < 80 mL/min/1.73m^2 indeed has an mGFR < 80 mL/min/1.73m^2. Of the 295 potential donors analysed (44% male, mean age 50 ± 11 years, BMI 27 ± 5 kg/m^2). Mean mGFR, MDRD, CKD-EPI and CG/BSA were 103 ± 16, 81 ± 15, 88 ± 14 and 95 ± 21 mL/min/1.73m^2 respectively. In 19 subjects mGFR was < 80 mL/min/1.73m^2, for MDRD this were 166, CKD-EPI 98 and CG/BSA 74 subjects. The predicted probabilities of mGFR < 80 when eGFR was < 80 mL/min/1.73m^2, were 8, 14 and 18% for MDRD study, CKD-EPI and CG/BSA equation. Analyses for subgroups defined by gender, age and BMI are shown in figure 4, showing that the prediction of true GFR < 80 is slightly improved by taking into account these demographic parameters.

Discussion

This paper discusses the influence of level of mGFR on performance of eGFR in a situation of normal and lower mGFR in the same healthy individual. When expressed in mL/min, bias of eGFR is reduced at lower values of mGFR, i.e. after kidney donation. For bias expressed as percentage difference, this was only true for CG/BSA. In the limited time frame, donor characteristics remained similar. The previously described determinants of bias: age, BMI and gender influenced bias, though only limited. Therefore, these data support the assumption that mGFR is a determinant of performance of eGFR.

This study is the first to compare performance of eGFR in high and lower ranges of mGFR within the same subjects. Although we can not exclude subtle changes in body composition, anthropometric parameters stayed virtually constant over this time frame, and age increased similarly for all subjects. Therefore, our setting allows dissecting the effect of mGFR as such.

For arithmetic calculated bias (mL/min), all equations showed improved performance post-donation. The relationship between eGFR and mGFR improved, although the difference for CKD EPI is markedly lower than for MDRD and CG/BSA (figure 2). Influence of mGFR on arithmetic bias was confirmed (figure 3).

For relative values (percentage difference), CG/BSA performance significantly improved post-donation, although influence of mGFR post-donation was less. MDRD study equation was stable in performance analysis, though mGFR influenced MDRD bias at both time-points. CKD-EPI performs slightly worse for post-donation values, reflected by a loss of influence of mGFR on bias (figure 3).

Although our data support an important role of the absolute level of GFR on performance of eGFR, we cannot ascribe deviation from mGFR in these healthy subjects to mGFR alone. Healthy people may differ in body composition and physiology of creatinine handling from CKD patients, leading to a more dominant effect of protein intake and muscle mass on serum creatinine in healthy subjects, whereas in CKD patients true GFR may have a more dominant effect (2,14). Comparing performance in healthy subjects with lower mGFR to matched CKD patients can give insight in this mechanism. Since both MDRD and CG equation were derived

from populations with a reduced GFR (9,10), they may overstate the strength of the relationship between GFR and serum creatinine in healthy subjects (14). The CKD-EPI equation was developed and validated in populations with a broader range of renal function and contains a spline function which enables it to differentially capture this relationship and therefore shows to be less influenced by mGFR in this study.

To account for variation in muscle mass as a variable in creatinine production, demographic and/or other anthropometric data have been included in GFR estimating equations.The MDRD algorithm was shown to inadequately represent variance in muscle mass (26), and produces systematic errors in estimation of GFR. Performance of CG was found to deviate with age and body mass index (16,25). Studies in renal transplant recipients confirmed age, gender and BMI, to be significant determinants of bias in MDRD study and CG equation (15,17,25).

Variation among laboratories in reporting serum creatinine has been proposed as an important cause of deviation of eGFR (11,24,27). We therefore used calibration equations to calibrate our serum samples to the Cleveland laboratory.

For MDRD Study and CG/BSA, influence of level of mGFR could be explained by the approximately hyperbolic curve of the relation between mGFR and serum creatinine, which translates to a stronger relationship between the equations and mGFR at lower levels of mGFR, here reflected by steeper slope of regression of eGFR on mGFR post-donation.

The new CKD-EPI equation seems to be more stable in different conditions. mGFR had less influence on group performance of CKD-EPI compared to the other two equations. In individual analysis CKD-EPI however showed a similar variation as the other equations. Bias of CKD-EPI was not influenced by age, BMI or gender.

All three equations significantly underestimated kidney function at both time points. This is in line with prior studies on the MDRD and CG equation. Therefore, recently the CKD EPI equation was proposed (12). Pre-donation values of 43 donors included in this study were used in the external validation set of the CKD EPI equation as well (12,19). In these 253 donors, CKD-EPI showed to be the most precise equation. Our current analysis does support better performance of the CKD/EPI equation in healthy kidney donors when compared to MDRD, albeit not optimal. CKD-EPI does show more stable performance than the other two equations which is an important characteristic for clinical use.

For donor screening the underestimation by eGFR at normal or higher values of mGFR can have large consequences, especially when taking in account that generally under 90% of results are even within +/- 30% of the measured result. Our Bayesian analysis illustrates large impact of the GFR-dependent underestimation of mGFR by eGFR in a population of prospective donors, where the prior probability of impaired renal function is low. For all three equations the probability that an eGFR <80 mL/min/1.73m^2 indicated an mGFR <80 ml/min/1.73m^2 was low. Thus, eGFR should be interpreted with extreme caution when used for donor screening purposes, lest a substantial proportion of subjects are declared unfit for donation based on a flawed renal function estimate.

In summary, level of mGFR is an important modulator of performance of eGFR. Whereas previously it was suggested that physiological differences in creatinine handling between subjects with a higher and lower GFR were the main cause of deviation of eGFR, we believe level of mGFR is important as well. Whereas for clinical use, use of eGFR has been discouraged at a value > 60 (7), epidemiological research tends to use eGFR above this level. We believe application of eGFR in the healthy should be used with caution. All three equations discussed here are an unreliable tool for evaluation of living kidney donors.

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Chapter 2

Performance of MDRD Study and CKD-EPI Equations for long term follow-up of non-diabetic patients with Chronic Kidney Disease

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Abstract

Background: Chronic kidney disease (CKD) typically extends over decades. Longitudinal monitoring of kidney function in CKD is thus of great importance. Here we retrospectively evaluate use of the MDRD Study and CKD-EPI equations to monitor long term course of kidney function, and to identify individuals with progressive kidney function loss.

Methods: Patients were selected from our outpatient clinic for having ≥4 GFR measurements (mGFR, ^{125}I-iothalamate) and ≥4 years follow-up. Renal function slopes were obtained by within-individual linear regression.

Results: 65 non-diabetic CKD patients (40 male, mean baseline age 44±12) with a median (range) of 9 (4-16) mGFR measurements and a median follow-up of 11 (4-33) years were included. Both equations significantly underestimated mGFR/BSA at baseline and end of follow-up. mGFR slope was significantly underestimated by MDRD Study, but not by CKD-EPI equation (slopes -1.41 ± 2.06, -1.07 ± 1.72 and -1.39 ± 1.77 mL/min/1.73m^2 per year respectively). Sensitivity and specificity to identify progressive kidney function loss (mGFR/BSA slope >1.5 mL/min/1.73m^2 per year, n = 23) were 78 and 88% for MDRD Study and 91 and 80% for CKD-EPI equation. In the subgroup of progressors both MDRD Study and CKD-EPI equation underestimated the rate of mGFR loss ($p<0.05$)

Conclusion: Long term course of mGFR is reasonably well estimated by CKD-EPI and slightly underestimated by MDRD Study equation. Patients with progressive kidney function loss may however not be reliably identified, so caution is warranted when using these equations in clinical practice.

Introduction

Reliable monitoring of kidney function over time is of major importance for the treatment and prevention of progressive kidney function loss. For simple assessment of kidney function, several creatinine-based kidney function equations have been developed. The Modification of Diet in Renal Disease (MDRD) Study equation (1,2) is most extensively used. Although this equation has proven its performance in patients with kidney impairment, performance in subjects with better kidney function (mGFR > 60 mL/min/1.73 m^2) is poor (3-10). For this reason the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (11) recently presented a new equation. This CKD-EPI equation was empirically developed from a large cross-sectional data set in different populations, including renal patients as well as healthy subjects and specifically pursues a better performance in the higher ranges of GFR.

For the clinical applicability of these equations in the management of CKD it is crucial that they provide a reliable estimate of the changes in kidney function over time over an extended period, as CKD typically evolves and progresses over decades. Their validation however, mainly relies on cross-sectional data. Longitudinal performance studies are sparse so far, and were mainly conducted in transplant recipients (12-17) and diabetic patients (18;19). In non-diabetic CKD patients no studies are available with a follow-up beyond 4 years (20-22).

In this study we aim to assess, first, the performance of the MDRD Study and CKD-EPI equations compared to gold standard kidney function measurement in the long term follow-up of non-diabetic CKD patients. Moreover, we studied their performance in detecting individuals with progressive kidney function loss.

Methods

We retrospectively evaluated data on kidney function of renal patients of the nephrology outpatient clinic of the University Medical Center Groningen. To ensure long term follow-up, we retrieved data from all patients enrolled in renal hemodynamic studies performed between 1972 and 1995 at our centre. Of these 156 patients, 99 had follow-up of renal function at our center, of which 15 had a renal transplant at baseline and were excluded from this study. We further selected patients for having a minimum of 4 GFR measurements and at least 4 years follow-up, giving 72 eligible patients. Seven patients were lost due to missing data on weight or length. Of the 65 patients enrolled in this study, 27 had essential hypertension, 17 membranous glomerulopathy, 5 focal glomerulosclerosis, 5 IgA nephropathy, 3 a single kidney after nephrectomy for reasons other than kidney donation, 2 polycystic kidney disease and 6 other diagnoses like ischemic lesions and Barrter syndrome. Patients with diabetes mellitus were excluded to preclude effects of temporary hyperfiltration on slope analysis. Follow-up was ended from the moment patients received renal replacement therapy or a kidney transplant to avoid effect on kidney function of dialysis and immunosuppressant drugs.

GFR measurement

Glomerular filtration rate (GFR) was measured by constant infusion of low-dose ^{125}I iothalamate as described by Apperloo et al. (23). Simultaneously, effective renal plasma flow is measured as the clearance of ^{131}I-hippurate. For the measurements subjects are seated in a quiet room, in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I-iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period follows, after which the clearance periods start. Clearances are measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer and P represents the tracer value in plasma at the end of each clearance period. GFR is calculated from U*V/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5% (23). Creatinine was determined from blood samples drawn at the start of the GFR measurement. This procedure was unaltered over the duration of the observation period.

Calculations

We used the abbreviated, four variable MDRD Study equation that was reexpressed for standardized serum creatinine (SCr) samples (2), which was calculated as follows:

MDRD = $175 * (SCr\ mg/dL)^{-1.154} * (age)^{0.203}$ (* 0.742 if female)

CKD-EPI equation was calculated gender specific, and stratified by creatinine levels. The following calculations were used (11):

Female with SCr ≤0.7 mg/dL: $GFR = 144 * (0.993)^{age} * (SCr/0.7)^{-0.329}$

Female with SCr > 0.7 mg/dL: $GFR = 144 * (0.993)^{age} * (SCr/0.7)^{-1.209}$

Male with SCr ≤ 0.9 mg/dL: $GFR = 141 * (0.993)^{age} * (SCr/0.9)^{-0.4111}$

Male with SCr > 0.9 mg/dL: $GFR = 141 * (0.993)^{age} * (SCr/0.9)^{-1.209}$

No correction for ethnicity was applied in both the MDRD Study and CKD-EPI equations, as none of the patients were of African ethnicity. From here, these equations are referred to as estimated GFR (eGFR). BSA was calculated as according to DuBois (24). mGFR was normalized by dividing the raw sample by BSA and multiplying it with 1.73, giving mGFR/BSA. The slope of kidney function loss was calculated by within-individual linear regression.

Analysis of predictive performance

Performance of MDRD Study and CKD-EPI equations against mGFR/BSA was analyzed as proposed by Bostom (25) and Stevens(26), presenting bias, precision, and accuracy. Bias was calculated as median of the absolute difference (mGFR/BSA - eGFR) and of the percentage difference ((mGFR/BSA – eGFR)/ mGFR/BSA * 100), giving a numeric or arithmetic value and a relative value. Precision represents the overall 'fit' of the new model against the gold

standard. It is represented by the interquartile range (IQR) of (mGFR/BSA - eGFR). Accuracy reflects the proportion of subjects with eGFR values within +/- 30% of mGFR/BSA (P_{30}).

Calibration of serum creatinine samples

Serum creatinine was measured by enzymatic assay on the Roche Modular in blood samples drawn after 1st March 2006. Before this date, samples had been measured by Jaffé alkaline picrate assay, on the MEGA, Merck KGaA, Darmstadt, Germany. Both methods were calibrated to the reference standard, i.e. Cleveland Clinic Laboratory measurements, as proposed by Coresh et al (27). To this purpose, a total of 516 blood samples with a broad range of creatinine were sent to the Cleveland Laboratory, of which 177 were from before March 1, 2006. Samples for calibration purposes were stored at -80°C until measured on the Roche <P> module Enzymatic assay with verified traceability to the reference standard IDMS. Calibration equations were as follows: calibrated serum creatinine = [-0.300 + 1.217 * (UMCG Jaffé creatinine values in mg/dL)] for measurements before 1st March 2006 and [0.011 + 1.087 * (UMCG Roche creatinine values in mg/dL)] for measurements after 1st March 2006. MDRD Study, and CKD-EPI equations were calculated from calibrated creatinine values.

Calculation of kidney function slope and definition of progressive kidney function loss

Individual slopes of kidney function loss were calculated by within-individual linear regression. To confirm linearity of individual kidney function slopes, we collected all creatinine samples available within the study period for each patient, and performed residual analysis on the individual creatinine slopes. Two patients had a non-linear creatinine slope, though mGFR slope was confirmed to be linear. As control of the abovementioned method, kidney function loss was also estimated by means of linear mixed effect models with random coefficients and random intercepts. Since both methods provided very similar estimates of mean slopes, within-individual linear regression was used for further analysis. To evaluate the performance of the equations to detect progressive kidney function loss, we identified “progressors”, defined as patients with a rate of renal function loss of at least two-fold higher than in the general population. In the Baltimore Longitudinal Study of Aging the normal age-related renal function decline was -0.75mL/min/year (28), so we classified a GFR decline > 1.5 mL/min/1.73m^2/year as progressive function loss. We tested the sensitivity and specificity of both equations to identify progressors. Additionally, we studied the predictive performance of the equations in the subgroup of progressors as described above.

Statistical analysis

Analyses were performed using SPSS software version 16.0, SAS version 9.1, Stata version 10.0, Microsoft Office Excel 2003, and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [inter quartile range (IQR)]. Paired Sample’s T Test and Wilcoxon Signed Ranks test were used to analyse differences between baseline and last observation values, and to analyse differences between mGFR/BSA and eGFR, and MDRD Study and CKD-EPI equations. Differences between groups were tested by independent

samples *t*-test, Mann Whitney U test and Kruskal Wallis test. Differences between accuracy were tested with Chi square.

For baseline and last observation values of MDRD Study and CKD-EPI equations, Bland Altman analyses were performed. Determinants of bias at baseline and end of follow-up and bias of slope were examined by backward linear regression.

Results

Data on kidney function measurements of 65 patients (42 male) were obtained for analyses. The median (IQR) number of GFR measurements was 9 [6-11] with a median follow-up time of 11 [7-18] years. Patient characteristics for baseline and last observation values are listed in table 1. CKD-EPI equation provided significantly higher values than the MDRD Study equation ($p<0.01$), but both equations significantly underestimated mGFR/BSA at both time points ($p<0.01$).

Table 2 compares the cross-sectional performance of the equations at baseline and end of follow-up. At baseline, bias of the MDRD Study equation, expressed in mL/min/1.73m^2 was

Table 1: Patient characteristics at baseline and end of follow-up.

	Baseline	Last observation	*P* value
Age (years)	45 ± 11	58 ± 13	<0.01
BMI (kg/m^2)	25.6 ± 3.7	26.4 ± 4.2	<0.01
Serum creatinine (mg/dL)	1.30 ± 0.52	1.84 ± 1.12	<0.01
mGFR/BSA (mL/min/1.73m^2)	78 ± 27	58 ± 29	<0.01
MDRD study equation (mL/min/1.73m^2)	63 ± 24*	47 ± 23*	<0.01
CKD-EPI (mL/min/1.73m^2)	70 ± 26*†	51 ± 25*†	<0.01

Data are expressed as mean ± standard deviation. * $p<0.01$ compared to mGFR value; † $p<0.01$ compared to MDRD stuy equation value.

Table 2: Overall performance of MDRD Study and CKD-EPI equations.

		Baseline		Last Observation	
		MDRD study	CKD-EPI	MDRD study	CKD-EPI
mL/min/1.73m^2	Bias	15 (7-19)	8 (1-13)†	9 (6-13)*	6 (4-9)†
	Precision	22 (0-32)	21 (-8-25)	16 (0-22)	16 (-3-19)
%	Bias	21 (11-28)	12 (2-21)†	20 (12-23)	12 (7-19)†
	Precision	28 (0-35)	31 (-10-29)	22 (-1-32)	23 (-10-27)
	P_{30} (%)	66	82†	77	82†

Bias values represent median (95% CI), precision values IQR (95% CI). * $p<0.05$ compared to baseline value; †$p<0.001$ compared to MDRD study equation value.

Table 3: Slopes of mGFR and MDRD Study and CKD-EPI equations.

	Slopes (mL/min/1.73m^2 per year)
mGFR/BSA	-1.5 ± 2.0
MDRD study	-1.1 ± 1.7*
CKD-EPI	-1.4 ± 1.8†

Data are expressed as mean ± standard deviation. * p< 0.05 compared to mGFR/BSA slope; † p<0.01 compared to MDRD study equation slope.

significantly larger than for the CKD-EPI equation (p<0.05). At the end of follow-up, bias of the MDRD Study equation had decreased significantly (p<0.05), whereas for the CKD-EPI equation it had remained stable. Expressed as percentage difference, bias was stable during follow-up for both equations. At both time-points, CKD-EPI had higher accuracy than the MDRD Study equation (all p<0.001). The biases of both the MDRD Study and the CKD-EPI equations were best predicted by mGFR/BSA (adjusted R^2=0.23 and 0.12 respectively, p<0.01 for baseline bias and adjusted R^2=0.47, and 0.25 p<0.01 for end of follow-up). In all analyses, age, duration of follow-up and 24 hour urinary creatinine excretion had no influence on bias (data not shown).

Slopes for change in mGFR/BSA and eGFR over time are shown in table 3. mGFR/BSA slope and CKD-EPI equation slope were not significantly different. Both slopes were significantly steeper than the MDRD Study equation slope (p<0.05). Figure 1 displays scatter plots for the regression of respectively the MDRD Study and CKD-EPI equation slope on mGFR/BSA slope. The CKD-EPI slope had a stronger relation with mGFR/BSA than the MDRD Study slope (R^2 0.52 and 0.45 respectively, p<0.01). Figure 2 displays the performance of eGFR/BSA slope by Bland Altman analysis: no systematic error was found for either of the equations.

Figure 3 displays distribution of within subject bias between MDRD Study and CKD-EPI equations slope and mGFR slope, and median mGFR/BSA slope values for the different

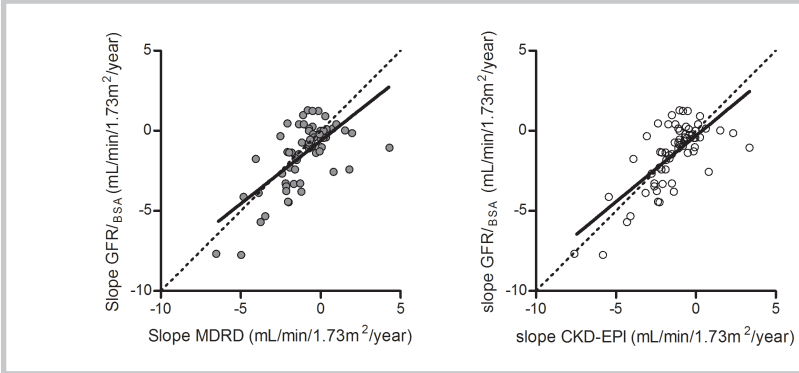

Figure 1: Scatter plots of regression of MDRD Study equation slope on mGFR/BSA slope (left panel) and CKD-EPI equation slope on mGFR/BSA slope (right panel). R^2 for MDRD Study and CKD-EPI equations are 0.52 and 0.45 (both p<0.05).

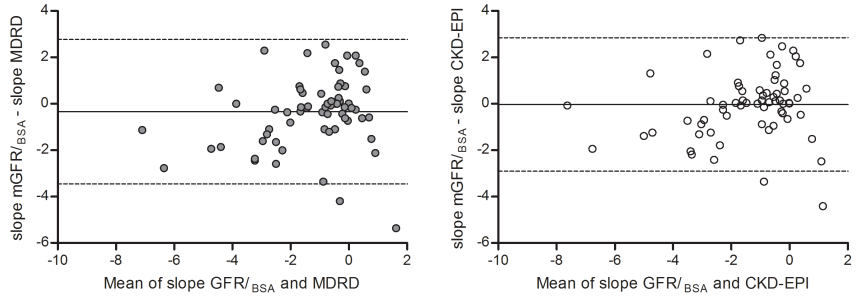

Figure 2: Bland Altman analysis of performance of MDRD Study equation slope against mGFR slope (left panel) and CKD-EPI equation slope against mGFR slope (right panel). Solid line represents mean bias, dotted lines represent ± 2 SD interval.

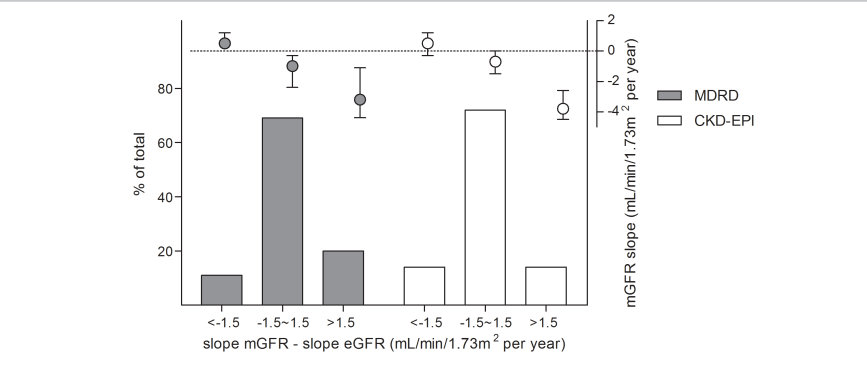

Figure 3: Distribution of bias of slope (mGFR/BSA slope – eGFR slope) for MDRD Study (left, grey bars) and CKD-EPI equations and median mGFR/BSA slope for the particular categories. Bars represent % of total (left y-axis), circles and error bars represent median (IQR) value of mGFR/BSA slope (right y-axis).

categories. The majority of subjects had bias between -1.5 and 1.5 mL/min/1.73m^2 per year, 69 and 72% for MDRD Study and CKD-EPI equations. Respectively 20 and 14% had bias above 1.5 mL/min/1.73m^2, and thus an eGFR slope more positive than mGFR/BSA slope. In 11 and 14% eGFR slope bias was below -1.5 mL/min/1.73m^2. For both equations, median mGFR/BSA slope decreased over the groups ($p<0.01$).

Next, we studied the performance of the equations in the detection of progressive kidney function loss. A mean loss of mGFR/BSA > 1.5 mL/min/1.73m^2/year was present in 23/65 patients, classified as progressors. Individual values for the slopes of mGFR and eGFR are given in figure 4 with a break-up by progressor-status. Sensitivity and specificity for detection of progression were 78 and 88% for the MDRD Study and 91 and 81% for the CKD-EPI equation. No differences in baseline patient characteristics, duration of follow-up, number of kidney function measurements, kidney function or 24 hour creatinine excretion were found

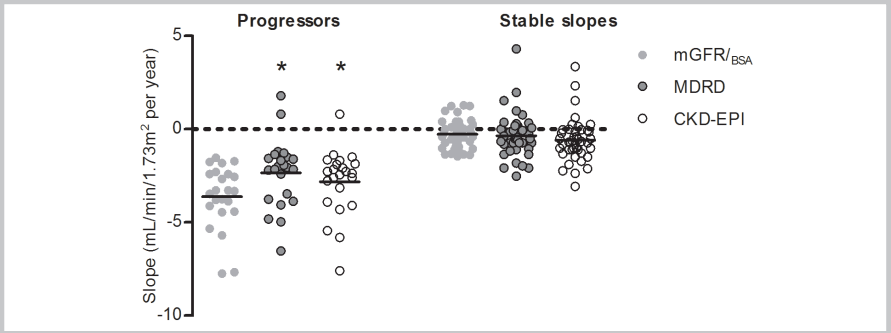

Figure 2: Bland Altman analysis of performance of MDRD Study equation slope against mGFR slope (left panel) and CKD-EPI equation slope against mGFR slope (right panel). Solid line represents mean bias, dotted lines represent ± 2 SD interval.

Table 4: Bias (mL/min/1.73m^2) of MDRD Study and CKD-EPI equations by break-up by rate of kidney function loss.

	Progressive slopes (n=23)			Stable slopes (n=42)		
	Baseline	Last observation	*P* value	Baseline	Last observation	*P* value
MDRD Study	22 (20-25)	20 (14-26)	22 (20-24)	17 (12-21)	0.16	73 (68-79)
CKD-EPI	14 (11-16)	18 (14-22)	13 (11-16)	16 (13-21)	0.20	89 (85-93)

Values represent median (95% CI).† p<0.01 compared to corresponding MDRD study equation value.

between patients correctly and wrongly classified as progressor (data not shown). Positive and negative predictive values for progression were 78 and 88% for the MDRD Study and 72 and 94% for the CKD-EPI equation. In progressors the slope of mGFR/BSA (-3.6±1.7 mL/min/1.73m^2/year) was significantly underestimated by both equations, with values of -2.4±1.8 and -2.8±1.8 ml/min/1.73m^2 for the MDRD Study and CKD-EPI equations respectively (p<0.05, figure 4), whereas in the stable subjects the slopes were similar for mGFR, MDRD Study and CKD-EPI equations: 0.3±0.8 vs. -0.4±1.2 and -0.6±1.2 mL/min/1.73m^2 per year, respectively. Baseline level of mGFR and both equations were similar between the groups (data not shown). Performance analysis showed that bias remained stable between baseline and the end of follow-up in the non-progressors, whereas in the progressors bias was significantly smaller at end of follow-up (table 4).

Discussion

This study evaluated the MDRD Study and CKD-EPI equations for long term follow-up of non-diabetic CKD-patients. The MDRD study equation underestimated kidney function decline, but the CKD-EPI equation more accurately quantified the mean rate of kidney function loss over time. The sensitivity and specificity of both equations to detect progressive kidney function loss were limited, and in progressors the rate of kidney function loss is

underestimated. This warrants caution in the application of the equations in the monitoring of kidney function in clinical practice.

Previous studies evaluating the performance of MDRD Study equation for longitudinal follow-up focused mainly on transplant recipients. In kidney (12-15), lung (16) and liver (17) transplant recipients, the MDRD Study equation had a reasonable performance on group level. However, it tended to underestimate the rate of kidney function loss and the number of patients developing kidney function impairment. Two studies in patients with type II diabetes mellitus showed underestimation of kidney function slope, with especially large underestimation in in early stages of nephropathy (hyperfiltration and normal kidney function) (18,19).

In our study, CKD-EPI equation performed well in the prediction of mean mGFR, while MDRD Study equation showed slight underestimation. However the sensitivity and specificity to detect individuals with progressive kidney function loss were limited. In particular, the positive predictive values were suboptimal, and in progressors, the rate of kidney function loss was underestimated. This is consistent with previous studies (13,16,18,19,22) where the MDRD Study equation underestimated kidney function loss as well, and did not reliably detect progressive function loss. In the original MDRD population Xie et al. evaluated longitudinal performance of the MDRD Study equation. In 542 patients with a follow-up of 2.6 years the mean rate of mGFR decline, being -3.9 mL/min/1.73m^2/year, was underestimated by the MDRD Study equation by some 28% (22). In the African American Study of Kidney Disease and Hypertension' (AASK) population, Lewis et al. showed that in their four year study period the AASK equation underestimated kidney function loss as well (-1.6 vs. -1.9 mL/min/1.73m^2 per year) (21).

Our data demonstrate that underestimation of mGFR slope is due to the change in bias over time, with less underestimation at the end of follow-up. This is more likely due to loss of kidney function rather than time-span as such, since it was only found in progressors. It is well established by cross-sectional studies that underestimation of mGFR by eGFR is smaller at lower absolute levels of mGFR, as also the case in our population. Recent studies by Lee and our own group, in predialysis patients and healthy kidney donors, respectively, showed that a within individual decrease in mGFR is associated with a decrease in bias as well (20,29). Inherent to this mGFR dependency of bias, eGFR bias decreases over time in subjects with progressive kidney function loss and thus eGFR slope will be less steep compared to mGFR slope. Other kidney function related factors, like diminished creatinine excretion, and altered muscle mass over time may further influence eGFR performance.

In our population the CKD-EPI equation performed better than MDRD Study equation, both cross-sectionally and longitudinally. It was less influenced by the level of mGFR/BSA, in line with its development and validation in datasets with a broad range in mGFR and a spline for creatinine. Therefore, bias is more stable over the range of kidney function, and accordingly the deviation of its slope from mGFR slope is stable over time as well.

What could be the implications of our findings? In clinical practice reliable monitoring of kidney function is important to assess long term prognosis, and accordingly allocation of preventive measures and timely referral for specialist care and renal replacement therapy.

The shortcomings of creatinine and the reciprocal of creatinine to this purpose are well-established (30). Our data show that eGFR has shortcomings for longitudinal monitoring as well. In particular the distinction between stable patients and progressors, which is essential for applicability in clinical practice, is hampered by the limited sensitivity and specificity of eGFR to detect progressive renal function loss, which occurred in spite of a prolonged observation period and multiple measurements. For individual patients, positive and negative predictive values are the relevant characteristics of a diagnostic test to consider, and these are strongly affected by the a priori probability of progression in the population. Thus, whereas the positive and negative predictive values for progression were rather acceptable in our university hospital population with one-third progressors, in the general population or in general practice identification of progressors will be blurred by the lower proportion of progressors. For instance, in a population with 10% progressors, the positive and negative predictive value for MDRD Study and CKD-EPI equations would be 42 and 97% and 35 and 99% respectively. This will result in an unwarranted number of falsely-positive identified progressors whereas on the other hand true progressors still escape from being detected. For a more severe definition for progression, i.e 2 mL/min/1.73m^2/year, the results were essentially similar (data not shown). This performance is reason for concern as regards the use of eGFR for follow up of renal function in general practice.

It should be noted that to evaluate kidney function slopes, often linearity is assumed. Although in this study all but two patients were shown to have a linear kidney function slope, this does not always apply. The two patients with the non-linear creatinine slope were not among the missed progressors.

The use of creatinine-based parameters as an outcome parameter in clinical trials in CKD has been criticized and it has been argued that hard end point studies would be preferable (31,32). However, this is not feasible for intervention studies in earlier stages of CKD. Accordingly, and supported by our current data, it has been argued (14) that reference methods should be used for monitoring kidney function in clinical trials.

Our study has several limitations, the most important being the relatively small sample-size, the mono centric character, and the lack of standardized timing of measurements. The conclusions do not apply to patients with African ethnicity, diabetic patients and kidney transplant recipients all of whom were not included. Due to our inclusion criteria of a minimum of 4 mGFR measurements and 4 year follow-up, subjects with an extreme progressive slope, reaching ERSD and the need of renal replacement therapy within this time span, were excluded. All this limitations hamper generalisability. Still, these data derived from clinical practice may have better applicability than data derived from clinical trials.

In conclusion, this study shows acceptable performance of CKD-EPI equation and slight underestimation of mean function loss by MDRD Study equation in long term follow-up of CKD-patients. Individual patients with more progressive kidney function loss might however be missed. Thus, caution is warranted in the application of the equations in the monitoring of kidney function in individual patients.

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Chapter 3

Performance of estimated GFR in longitudinal follow-up of living kidney donors

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In preparation

Abstract

Background: Due to more liberal selection criteria, living kidney donors nowadays are more marginal. This emphasizes the need for proper and accurate kidney function follow-up in former donors, preferably by simple methods. This study evaluates the use of creatinine-based estimating GFR equations for living donor follow-up.

Methods: Included were 132 consecutive living kidney donors. All had GFR (^{125}I-iothalamate) measured prior to, and two months and 5.4 ± 1.5 years post-donation. GFR was estimated by MDRD Study, Cockcroft-Gault (CG) and CKD-EPI equations. GFR decline exceeding 1.0 mL/min/1.73m^2 per year was classified as a progressive kidney function slope.

Results: Over donation, GFR fell from 104 ± 15 to 67 ± 11, and then increased to 73 ± 11 mL /min/1.73m^2 at long term post-donation. The equations significantly underestimated GFR at all time points. The equations showed moderate performance to estimate mean GFR slope (1.16 ± 1.4 mL/min/1.73m^2 per year), although the difference from the GFR slope was larger for CG equation (0.89 ± 1.72) than for MDRD study (1.03 ± 1.43) and CKD-EPI equation (1.03 ± 1.66). Thirteen donors had a progressive GFR slope (median[IQR] slope -1.3 [-1.4 ~ -1.2] mL/min/1.73m^2 per year). In these donors, all equations showed a positive median slope.

Conclusion: In conclusion, MDRD Study and CKD-EPI equation perform moderately at group level to estimate long term GFR slope in former kidney donors. All equations, however, have low capacity to detect individual donors with a negative, progressive kidney function slope.

Introduction

Living kidney donation is of major importance for kidney transplantation programs nowadays. Over the last years, selection criteria have become less strict (1;2), enabling more marginal donors to enroll in the screening process and donate a kidney. This strengthens the need for thorough screening, with reliable kidney function measurements. Furthermore, donor follow-up has become more important to ensure long term donor safety.

Since gold standard glomerular filtration rate (GFR) measurement is laborious and expensive, creatinine-based equations providing estimates of GFR (eGFR) are most often used for donor screening and post-donation follow-up. The most widely used equations, the Modification of Diet in Renal Disease (MDRD) study equation and Cockcroft Gault (CG) equation, however, are known to perform poorly in subjects with normal or only mildly impaired kidney function (3-9), and several studies reported them inappropriate for use in living donor screening (6;8;10-13). The more recently developed Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation performs better at higher levels of kidney function, and, hence, performs more stable before and early after kidney donation (14). For long term post-donation follow-up, however, no data are available yet. Most data on predictive performance of creatinine-based equations in general were obtained cross-sectionally, and performance for longitudinal follow-up is less well documented. A limited number of studies in CKD patients tested longitudinal performance of eGFR equations, and found reasonable performance on group level. However, the rate of kidney function loss was underestimated by all tested equations (15-20), and the sensitivity and specificity to detect progressive function loss were not optimal.

For former kidney donors, longitudinal data are lacking altogether. Therefore, in the current study, we analyzed the performance of three estimating equations, as compared to gold standard GFR measurement, for long term follow-up of former living kidney donors.

Methods

In this study, 132 consecutive living kidney donors were evaluated. All donated between 1984 and 2006 in the University Medical Center Groningen. GFR was measured (mGFR) four months prior and two months following donation as part of the screening program and early post-donation evaluation. Since 2007, all former donors in our centre are invited for a second post-donation kidney function measurement five year post-donation. Donors who donated before 2002 were all invited in one follow-up round. For inclusion in the current analysis, we selected all donors for whom complete data on all three time points were available. At long term follow-up, mean duration since donation was 5.4 ± 1.5 year, with a range of 2.0 to 11.6 years. Procedures were conducted in accordance with the Helsinki declaration.

Measurements

GFR was measured by constant low-dose infusion of the radio-labeled tracer ^{125}I iothalamate, as originally described by Donker, and more recently by Visser et al. (21-23). Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. For the measurements, subjects were seated in a quiet room in, in a semi-supine position. After

drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution, (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra 0.6 MBq of ^{125}I iothalamate, was given, followed by constant infusion at twelve mL/h. To attain stable plasma concentrations of both tracers, a two hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next two hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer, and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%.

In our centre, before 1st March 2006, creatinine was measured by Jaffé alkaline picrate assay, on the MEGA, Merck KGaA, Darmstadt, Germany. Thereafter, creatinine was measured by enzymatic assay on the Roche Modular. Both methods were calibrated to the reference standard, i.e. Cleveland Clinic Laboratory measurements, as described before(12).

Calculations

Estimated GFR by MDRD Study (24;25), and CG (26) equations were calculated as follows, with serum creatinine (SCr) and body weight in mg/dL and kg.

MDRD = $175 * (SCr)^{-1.154} * (age)^{0.203}$ (* 0.742 if female).

Cockcroft-Gault = (140 – age) * body weight/(72 * SCr) (* 0.85 if female).

CKD-EPI equation was calculated gender specific, and stratified by creatinine levels (14). The following calculations were used:

Female with SCr ≤0.7: GFR = $144 * (0.993)^{age} * (SCr / 0.7)^{-0.329}$

Female with SCr > 0.7: GFR = $144 * (0.993)^{age} * (SCr / 0.7)^{-1.209}$

Male with SCr ≤ 0.9: GFR = $141 * (0.993)^{age} * (SCr / 0.9)^{-0.4111}$

Male with SCr > 0.9: GFR = $141 * (0.993)^{age} * (SCr / 0.9)^{-1.209}$

Both for the MDRD Study and CKD-EPI equations, no correction for ethnicity was applied as none of the donors were African Americans.

BSA was calculated as according to DuBois (27). mGFR and CG were normalized by dividing the raw sample by BSA and multiplying it with 1.73, giving mGFR/BSA and CG/BSA. Mean arterial pressure (MAP) was calculated as: MAP = (1/3 [systolic pressure - diastolic pressure] + diastolic pressure). Kidney function slopes were calculated as: slope = ([long term value – early post-donation value]/ duration of follow-up); giving a slope in mL/min/1.73m^2 per year.

Analysis of predictive performance

Performance of MDRD Study and CKD-EPI equations against mGFR/BSA was analyzed as proposed by Bostom (28) and Stevens (29), presenting bias, precision, and accuracy. Bias was calculated as the median difference (mGFR/BSA - eGFR) and of the percentage difference ((mGFR/BSA – eGFR)/ mGFR/BSA * 100), giving a numeric or arithmetic value and a relative value. Precision represents the overall 'fit' of the new model against the gold standard, and is represented by the interquartile range (IQR) of (mGFR/BSA - eGFR). Accuracy reflects the proportion of subjects with eGFR values within +/- 30% of mGFR/BSA (P30). To detect any

systematic error, the level of eGFR slopes were plotted against the difference between the mGFR/BSA slope and eGFR slope as described before by Stevens at all (29).

The main reason to monitor kidney function after donation is to detect possible kidney function loss over time. In the Nijmegen Biomedical Study, normal age-related kidney function decline of the Dutch population was -0.4 mL/min/1.73m^2 peryear (30). Thus, we classified a mGFR/BSA decline twice as fast, i.e. > 1 mL/min/1.73m^2 peryear, as a progressive kidney function slope.

Statistical analysis

Analyses were performed using PASW Statistics version 18 and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [IQR]. Independent samples *t*-Test, Mann-Whitney test and chi-square test were used to analyse for differences between groups. Differences within groups were tested with paired samples *t*-Test and Wilcoxon Signed Ranks Test.

Results

Donor characteristics and measured and estimated kidney function prior to and post-donation are shown in table 1. After nephrectomy, mGFR/BSA fell to 64 ± 11% of the pre-donation value early post-donation. At long term, mean GFR increased to 70 ± 11% of the pre-donation value. Nineteen donors had a mGFR/BSA <60 mL/min/1.73m^2 at long term. Kidney function course by measured and estimated GFR is shown in figure 1. With exception of the CG/BSA value early post-donation, all three estimating equations significantly underestimated mGFR/BSA at all time points (all p<0.01 compared to mGFR/BSA values, table 1).

Performance of the equations at the two post-donation time points is displayed in figure 2. For all equations, bias, relative bias, accuracy and precision were similar at both time points.

Table 1: donor characteristics prior to and early and long term post-donation.

	Pre-donation	Early post donation	Long term post-donation
Duration follow-up (years)	-0.5 ± 0.6	0.2 ± 0.0	5.4 ± 1.5
Age (years)	48 ± 11	49 ± 11	54 ± 11
BMI (kg/m^2)	26 ± 4	27 ± 4	27 ± 4
MAP (mmHg)	91 ± 9	92 ± 9	93 ± 9
Serum creatinine (mg/dL)	0.9 ± 0.2	1.3 ± 0.3	1.2 ± 0.2
mGFR/BSA (mL/min/1.73m^2)	104 ± 15	67 ± 11	73 ± 11
Estimated GFR			
MDRD Study (mL/min/1.73m^2)	81 ± 15*	50 ± 9*	55 ± 10*
CG/BSA (mL/min/1.73m^2)	96 ± 19*	63 ± 13	68 ± 15*
CKD-EPI (mL/min/1.73m^2)	89 ± 14*	55 ± 12*	60 ± 12*

Data are expressed as mean ± standard deviation. * p<0.01 *vs*. GFR/BSA value (paired samples *t*-test).

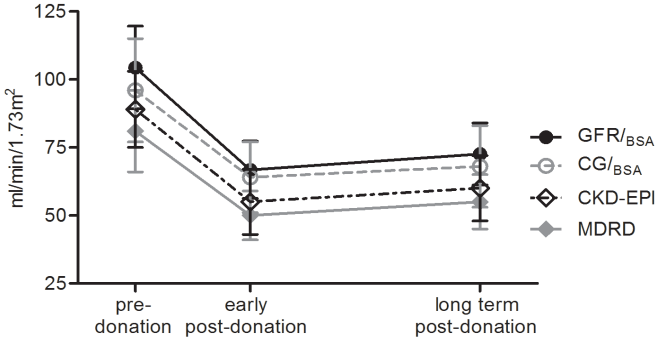

Figure 1: Kidney function course determined by mGFR and eGFR equations. Except for CG/BSA prior to donation, GFR/BSA was significantly underestimated by all three equations at all time points ($p<0.01$, paired samples *t*-test).

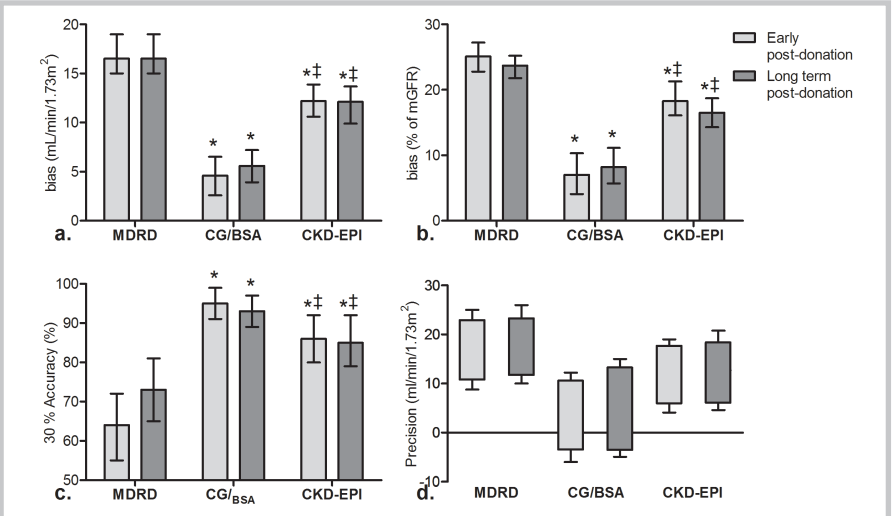

Figure 2: Performance of MDRD Study, CG/BSA and CKD-EPI equations. Figures display a) bias (mGFR-eGFR); b) bias as percentage of mGFR and c) 30% accarucy. * $p<0.05$ *vs*. corresponding MDRD value; ‡ $p<0.05$ *vs*. corresponding CG/BSA value (paired samples *t*-test and Chi^2 test).

CG/BSA had the lowest bias, relative bias and highest accuracy, followed by CKD-EPI equation.

Table 2 shows the mean kidney function slopes in measured and estimated GFR, calculated from the first and second post-donation time point for all donors (top panel). The average measured GFR increased with 1.16 ± 1.4 mL/min/1.73m^2 per year over this period, indicating an appropriate compensatory rise in kidney function. The mean slopes calculated from the equations were numerically less steep than the slope of mGFR/BSA but the differences did not reach statistical significance. The slopes of MDRD study and CKD-EPI

Table 2: Kidney function slopes per year for mGFR and the eGFR equations, for all donors (top panel) and for the subgroup of donors with a kidney function slope < -1 mL/min/1.73m^2 per year.

	Slope (mL/min/1.73m^2 per year)	*P* value
All donors (N=132)		
mGFR/BSA	1.16 ± 1.4	-
MDRD Study	1.03 ± 1.43	0.38
CG/BSA	0.89 ± 1.72	0.12
CKD-EPI	1.03 ± 1.66	0.40
Donors with kidney function slope < -1 ml/min/1.73m2 per year (N=13)		
mGFR/BSA	-1.3 [-1.4 – -1.2]	-
MDRD Study	0.6 [-0.1– 1.4]	<0.01
CG/BSA	0.5 [-0.2– 1.3]	<0.01
CKD-EPI	0.6 [-0.3 – 1.4]	<0.01

Values represent mean ± SD or median [IQR]. p-value represents comparison to GFR/BSA slope (paired samples *t*-test, and Wilcoxon Signed Ranks test).

equations however were significantly different from the CG/BSA slope (p=0.03 and 0.02 respectively).

The lower panel of Table 2 shows renal function slopes from a subgroup analysis in donors with a negative slope indicating renal function loss exceeding the anticipated age-related renal function decline between the first and second time point. This was defined as an mGFR/BSA decline larger than 1 mL/min/1.73m^2 per year. This occurred in thirteen donors, in whom the average rate of renal function decline was -1.3 ml/min/1.73m^2 per year. There were no differences in baseline age, BMI, blood pressure or kidney function between these donors and the rest of the population. mGFR/BSA at long term post-donation was lower (65 [58-68] vs. 73 [66-80] mL/min/1.73m^2, p < 0.01). Slopes for mGFR/BSA and the three estimating equations are shown in table 2. The three equations all significantly underestimated measured kidney function decline in this subgroup. Furthermore, in a substantial proportion of these donors with a negative mGFR/BSA slope, the slopes based on equations were positive, amounting to 9/13 donors for the MDRD Study and CKD-EPI equations, and in 8/13 donors for CG/BSA equation, indicating that the equations cannot reliably identify subjects with renal function loss.To detect systematic error, the slopes of all three equations were plotted against the difference in slope between mGFR/BSA and the equation (figure 3). All three equations show a systematic error, with overestimation of negative slope, implicating that renal function loss is assessed as being less severe in subjects with the steepest rate of renal function loss, and underestimation of positive slopes, implicating that the compensatory rise in renal function after donation is underestimated. By correlation analysis we aimed to identify determinants of the bias of the slopes, but no significant determinants of bias could be identified.

Discussion

This study evaluates the use of MDRD study, CG and CKD-EPI equations for long term post-donation follow-up of former living kidney donors. Overall, donor kidney function at long term was at a good level, and most donors showed a stable or positive kidney function course post-donation. Mean mGFR slope was reliably estimated by MDRD Study and CKD-EPI equation. Nevertheless, donors with a steep kidney function decline were missed by all three equations.

Studies reporting outcome of former kidney donors all show reassuring results with preserved kidney function at long term and a low risk for the development of CKD (31-37). Due to a more liberal donor selection, current donor populations, however, have a less favorable renal risk profile regarding age, overweight and blood pressure. The current donor population may, therefore, be at a higher risk for kidney function loss and impairment post-donation. This stresses the need for proper and accurate follow-up of former kidney donors. Previous studies on the use of eGFR in kidney donors focused mainly on cross-sectional performance of eGFR, applied for screening of potential donors or for post-donation kidney function assessment. Our current study is the first to evaluate the performance of eGFR for longitudinal follow-up over several years. Performance on group level appeared to be moderately well, but in the small subset of donors with a negative kidney function slope, all eGFR slopes diverged from mGFR/BSA slope, and failed to identify subjects with progressive kidney function loss. These findings are in line with prior studies on longitudinal use of eGFR in diabetic (15;18) and non-diabetic CKD-patients (16;17;19;20). In these studies, longitudinal performance was acceptable in subjects with an mGFR <60 mL/min (15;16), but poor in subjects with higher GFR (15;16;18-20).

All donors studied here had sufficient kidney function at long term. None of the donors developed kidney function impairment. A recent study by Fehrman-Ekholm et al. showed that post-donation kidney function may increase up to 15 years post-donation (38). The donors studied here, however, were strictly selected for having a pre-donation GFR > 80 ml/min. Thus, this is a highly selected population with subsequently good kidney function post-donation. Thirteen donors showed an increased rate of kidney function loss. All three equations failed to identify the increased rate of kidney function loss in these donors. Since many nowadays population are less strictly screened, and have more comorbidities than the population presented here, the risk for kidney function loss at long term may be increased. Thus, the capacity of the equations to detect rapid function loss becomes increasingly important. The performance of the equations in this study on identifying subjects with rapid function loss, therefore, is alarming.

Limitations of this study are the relative short duration of follow-up and the fact that only two kidney function measurements were performed post-donation. Thus, the kidney function slopes presented here were only based on two time points. Since the first kidney function measurement was early post-donation, the compensatory increase was not complete. Only three donors, however, had an increase in creatinine >0.05mg/dL between one and five year post-donation (data not shown). Thus, although the use of this early time point may misrepresent the compensatory grow, most donors had a stable or positive slope up to five year post-donation. Furthermore, we would like to mention our donors represent a highly selected population, with a pre-donation kidney function > 80 mL/min. Furthermore,

only 10% of donors was above 60 years of age, 17% was obese and 10% used antihypertensive drugs prior to donation. Our data, however, are in line with previous cross sectional studies in kidney donors, and with longitudinal studies in other populations.

What are the implications of our study for kidney function measurement in living donor follow-up? At group level, the performance is acceptable. However, the poor sensitivity and specificity to detect negative slopes is a reason for concern. It is in line with recent findings in CKD, supporting its robustness. Although the donors studied here all have good post-donation kidney function and on average a stable or positive kidney function slope, the current donor population has a less favorable renal risk profile, due to the more liberal donor selection regarding age, overweight and blood pressure (1;2). The current donor population may, therefore, be at a higher risk for kidney function impairment post-donation than subjects who donated a kidney previously, and, thus, proper and accurate follow-up is of main importance in the current era. Our data indicate that creatinine-based equations are not entirely reliable to detect post-donation kidney function loss and that their results should be interpreted with appropriate caution.

In summary, this study shows acceptable performance of MDRD study and CKD-EPI equation to estimate post-donation kidney function slope in former kidney donors. All equations, however, have low capability to detect individual donors with a negative kidney function slope. This raises questions for the suitability of estimating equations for donor follow-up, especially in the current era with more marginal donors. If gold standard kidney function measurement is not available, estimating equations should be used with caution.

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Part two:
Effect of donor risk profile on donor renal outcome

Chapter 4

Effects of preexistent hypertension on blood pressure and residual renal function after donor nephrectomy

4

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Abstract

Background: Living kidney donor selection has become more liberal with acceptation of hypertensive donors. Here we evaluate short-term and one and five year renal outcome of living kidney donors with pre-existent hypertension.

Methods: We compared outcome of hypertensive donors to sex, age and BMI matched control donors. Hypertension was defined as pre-donation antihypertensive drug use. All donors had GFR (^{125}I-iothalamate) and ERPF (^{131}I-hippuran) measured four months prior to and two months post-donation. A subset of donors had serum creatinine measured one year post-donation or a renal function measurement five year post-donation.

Results: Included were 46 hypertensive donors and 94 control donors (both 53% male, mean age and BMI 57±7 years and 28±4 kg/m^2). Pre- and early post-donation, systolic blood pressure and MAP were significantly higher in hypertensive donors. Control donors showed a rise in diastolic blood pressure post-donation, and thus the pre-donation difference was lost post-donation. Both at one year (29 hypertensive donors, 58 controls) and five year post-donation (13 hypertensive donors and 26 controls) blood pressure was similar. Renal function was similar at all time-points.

Conclusion: In summary, hypertensive living kidney donors have similar outcome in terms of blood pressure and renal function as control donors, early and one and five year post-donation.

Introduction

The number of patients reaching end stage renal disease and, thus, in need for renal replacement therapy has increased over the past decades. Due to both a shortage in deceased donor organs and the recognition of superior results, living kidney donors have become more important. To enlarge the donor pool, selection criteria for potential donors have become less strict, leading to an older and more overweight donor population (1). Many centers extended their criteria further and accept potential donors with well-regulated hypertension as well. This situation raises a new set of issues. Hypertension is a known risk factor for renal disease. Furthermore, previous studies have shown an increase in blood pressure post-donation in non-hypertensive donors (2-5). Nevertheless, longitudinal studies have shown that living kidney donors are not at increased risk of developing hypertension post-donation compared to the general population. However, little is known about the post-donation course of blood pressure of donors with pre-existent hypertension. Moreover, it is unknown whether hypertensive donors are at increased risk of impaired residual renal function post-donation. Textor et al. have shown that at one year post-donation, the presence of pre-existent hypertension has no adverse effects on blood pressure or GFR (6). Data on effective renal plasma flow (ERPF) and filtration pressure are, however, not available.

Here we evaluate short-term and one and five year outcome in terms of blood pressure and renal function of living kidney donors with pre-existent hypertension, compared to matched control donors.

Methods

In this study, 49 consecutive living donors with pre-existent hypertension and 98 matched controls were included. Controls were matched by gender, age and BMI. All donors donated at the University Medical Center Groningen between 1998 and 2010. Hypertension was defined as antihypertensive drug use pre-donation. Donors were found eligible to donate with a well-regulated blood pressure achieved by a maximum of two antihypertensive drugs. Blood pressure could was not allowed to exceed 145/85 mmHg at repeated measurements and/or ambulatory blood pressure measurement. Kidney function was measured as described below, four months prior to and two months after donation. In the abovementioned period, a cutoff of 80 mL/min of true GFR was used for acceptation of a potential donor in our center. There was no absolute upper limit for donor age, an upper limit for BMI has been set at 30 kg/m2 at 2008. Beforehand, no upper limit for BMI was used. Furthermore, proteinuria exceeding 0.5g/24h, or signs or end organ damage due to hypertension such as left ventricular hypertrophy, led to rejection of potential donors. One year after donation, donors came for an outpatient visit without measurement of GFR. Data were available for 29 subjects and 48 controls. For 13 subjects and 26 controls, five year follow-up, with kidney function measurement, was available as well. Procedures were conducted in accordance with the Helsinki declaration.

Kidney function measurements

Glomerular filtration rate (GFR) was measured by constant low-dose infusion of the radio-

labelled tracer ^{125}I iothalamate, as originally described by Donker, and more recenly by Visser et al. (7-9) Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. For the measurements, subjects were seated in a quiet room in, in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution, (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra 0.6 MBq of ^{125}I iothalamate, was given, followed by constant infusion at twelve mL/h. To attain stable plasma concentrations of both tracers, a two hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next two hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer, and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%. Next to basal kidney function, renal reserve capacity was measured pre- and two months post-donation as part of the screening and early follow-up. To obtain reserve capacity, the above-mentioned baseline procedure was extended for two hours. During this period, dopamine was infused at a rate of 1.5 µg/kg per minute. At five year post-donation, reserve capacity was not measured. Blood pressure was measured for thirty minutes during the kidney function measurement, in rest and semi-supine positions, with a semi-automated device (Dinamap® 1846; Critikon Inc, Tampa, FL, USA).

Calculations

Filtration fraction (FF) was calculated as the ratio of GFR and ERPF; FF = ([GFR/ERPF]*100). Mean arterial pressure (MAP) was calculated as MAP = (1/3[systolic blood pressure – diastolic blood pressure] + diastolic blood pressure). Renal reserve capacity was calculated as the response in renal function to dopamine (ΔGFR_{DOPA} and $\Delta ERPF_{DOPA}$) as: (stimulated renal function – basal renal function). Since at the out-patient visit one year post-donation no kidney function measurement was performed, we estimated GFR from serum creatinine by use of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (10). The following calculations were used (serum creatinine (SCr) in mg/dL):

Female with SCr ≤0.7 mg/dL: $GFR = 144 * (0.993)^{age} * (SCr / 0.7)^{-0.329}$

Female with SCr > 0.7 mg/dL: $GFR = 144 * (0.993)^{age} * (SCr / 0.7)^{-1.209}$

Male with SCr ≤ 0.9 mg/dL: $GFR = 141 * (0.993)^{age} * (SCr / 0.9)^{-0.4111}$

Male with SCr > 0.9 mg/dL: $GFR = 141 * (0.993)^{age} * (SCr / 0.9)^{-1.209}$

Statistical analysis

Analyses were performed using PASW Statistics version 18 and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [IQR]. Independent samples *t* Test, Mann-Whitney test and chi-square test were used to analyse for differences between groups. Differences within groups were tested with paired samples *t*-test and Wilcoxon Signed Ranks Test.

Table 1: Donor characteristics prior to and two months post-donation. The bottom part shows five year post-donation values for a smaller subset of donors. Characteristics prior to and early post-donation of this smaller group were similar as presented below.

	Hypertensive donors	Control donors	*P* value
N (% female)	47 (47)	94 (47)	1.00
Age at donation (years)	57 ± 7	56 ± 7	0.71
BMI at donation (kg/m^2)	28 ±3	28 ± 3	0.58
Pre-donation			
Systolic bp (mmHg)	139 ± 16	129 ± 12	<0.01
Diastolic bp (mmHg)	82 ± 10	77 ± 8	<0.01
MAP (mmHg)	101 ± 11	94 ± 8	<0.01
GFR (mL/min)	118 ± 19	113 ± 25	0.23
ERPF (mL/min)	419 ± 87	406 ± 91	0.45
FF (%)	29 ± 4	28 ± 4	0.40
ΔGFR_{DOPA} (mL/min)	10 ± 10	9 ± 11	0.96
$\Delta ERPF_{DOPA}$ (mL/min)	95 ± 70	92 ± 56	0.81
Urinary protein excretion (g/24h)	0.1 [0.0-0.3]	0.1 [0.0-0.2]	0.42
Two months post-donation			
Systolic bp (mmHg)	135 ± 13	128 ± 12	<0.01
Diastolic bp (mmHg)	81 ± 9	80 ± 9	0.36
MAP (mmHg)	99 ± 9	96 ± 9	0.03
GFR (mL/min)	73 ± 13	70 ± 13	0.21
ERPF (mL/min)	267 ± 48	263 ± 53	0.61
FF (%)	28 ± 3	27 ± 3	0.30
ΔGFR_{DOPA} (mL/min)	1.3 ± 4.1	1.8 ± 4.0	0.29
$\Delta ERPF_{DOPA}$ (mL/min)	38 ± 32	35 ± 22	0.68
Urinary protein excretion (g/24h)	0.0 [0.0-0.2]	0.0 [0.0-0.2]	0.45
Five year post-donation			
N	13 (46)	26 (46)	1.00
Systolic bp (mmHg)	137 [132-143]	131 [121-136]	0.07
Diastolic bp (mmHg)	80 [74-90]	77 [74-84]	0.50
MAP (mmHg)	102 [90-106]	94 [90-101]	0.27
GFR (mL/min)	81 [72-94]	78 [64-95]	0.53
ERPF (mL/min)	258 [250-299]	258 [223-306]	0.74
FF (%)	30 [29-33]	31 [28-32]	0.74
Urinary protein excretion (g/24h)	0.0 [0.0-0.1]	0.0 [0.0-0.2]	0.26

Values represent mean ± SD; median [IQR] or n (%). bp: blood pressure; ΔGFR_{DOPA}: stimulated GFR – basal GFR; $\Delta ERPF_{DOPA}$: stimulated ERPF – basal ERPF.

Results

Donor characteristics prior to and two months post-donation are shown in table 1. There was no difference in mean age or BMI at donation, reflecting a good match between hypertensive and control donors. Despite the use of in mean 1.3 ± 0.7 antihypertensive drugs, hypertensive donors had higher blood pressures prior to donation (all p<0.01). Post-donation, control donors showed a significant increase in diastolic blood pressure (p<0.05) while hypertensive donors had stable blood pressure. The difference in diastolic blood pressure was, thus, lost post-donation. Figure 1a shows the change in blood pressure from pre- to early post-donation. In hypertensive donors, no rise in blood pressure occurred, while the control donors showed a significant rise in diastolic blood pressure and non-significant rise in MAP. Basal renal function and renal reserve capacity were similar both pre- and post-donation, as well as urinary protein excretion. None of the donors had urinary protein excretion exceeding 0.5 g/24h. Figure 2a displays pre- and post-donation renal function graphically. The change in renal function of hypertensive donor parallels the change of control donors.

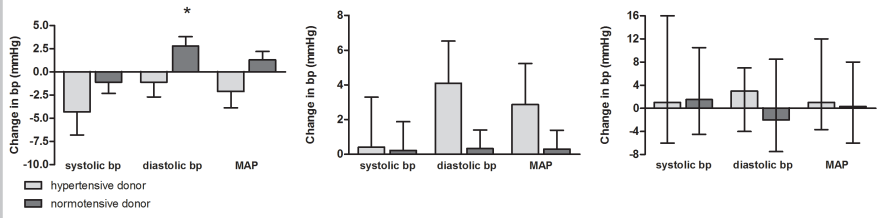

Figure 1: Changes in donor blood pressure over time. Figures display blood pressure from pre-donation to two months post-donation (left, n=141); from two months post-donation to one year post-donation (middle, n=87); from two months post-donation to five year post-donation (right). Bars represent mean ± SEM, and for the right grapgh median [IQR]. bp: blood pressure; * p<0.05 vs. hypertensive donors.

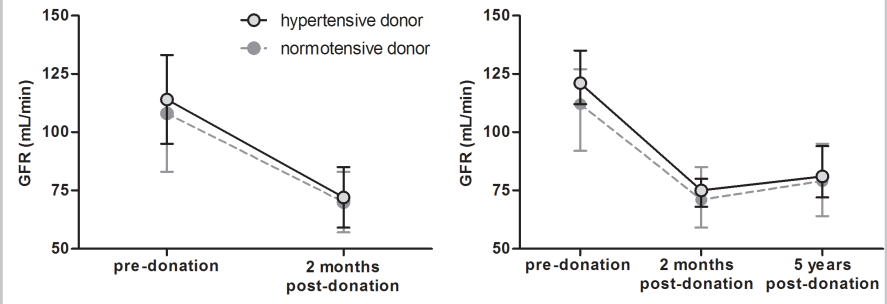

Figure 2: Change in GFR over time. Figures display pre- and two months post-donation GFR for the whole group (left, n=141), values represent mean ± SD; pre- and two months and five year post-donation GFR for a subset of donors (right, n=39), values represent median [IQR].

Table 2: Use of antihypertensive medication prior to and two months and one or five year post-donation. There were no differences in pre- and early post-donation drugs use between donors available and unavailable for one and five year post-donation follow-up.

	Hypertensive donors	Control donors
Pre-donation (n)	47	94
Number of donor using AH drugs	47 (100)	0 (0)
Number of AH drugs	1.3 ± 0.7	0 ± 0
Use of ACEi/ARB	27 (57)	0 (0)
Use of β-blocker	19 (40)	0 (0)
Use of calcium channel blocker	8 (17)	0 (0)
Use of diuretic	9 (19)	0 (0)
Two months post-donation (n)	47	94
Number of donor using AH drugs	42 (89)	0 (0)
Number of AH drugs	1.3 ± 0.5	0 ± 0
Use of ACEi/ARB	26 (55)	0 (0)
Use of β-blocker	15 (32)	0 (0)
Use of calcium channel blocker	7 (15)	0 (0)
Use of diuretic	12 (26)	0 (0)
One year post-donation (n)	29	58
Number of donor using AH drugs	17 (59)	1 (2)
Number of AH drugs	0.8 ± 0.8	0 ± 0.1
Use of ACEi/ARB	10 (34)	0 (0)
Use of β-blocker	8 (28)	0 (0)
Use of calcium channel blocker	2 (7)	1 (2)
Use of diuretic	4 (14)	0 (0)
Five year post-donation (n)	13	26
Number of donor using AH drugs	12 (92)	4 (15)
Number of AH drugs	2 [1-3]	0 [0-0]
Use of ACEi/ARB	8 (62)	2 (8)
Use of β-blocker	7 (54)	2 (8)
Use of calcium channel blocker	2 (15)	0 (0)
Use of diuretic	8 (62)	0 (0)

Values represent mean ± SD; median [IQR] or n (%). AH drugs: antihypertensive medication; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers.

Use of antihypertensive drugs is shown in table 2. On average, hypertensive donors used 1.3± 0.5 antihypertensive drugs, preferably an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB). The mean number of antihypertensive drugs did not increase over time, though there was a slight shift to more use of diuretics. Furthermore,

Table 3: Donor characteristics prior to and two months and one year post-donation.

	Hypertensive donors	Control donors	*P* value
N (% female)	29 (41)	58 (41)	1.00
Age at donation (years)	58 ± 6	57 ± 7	0.81
Pre-donation			
Systolic bp (mmHg)	138 ± 18	130 ± 12	0.02
Diastolic bp (mmHg)	80 ± 10	77 ± 8	0.09
MAP (mmHg)	100 ± 12	95 ± 8	0.03
Serum creatinine (mg/dL)	0.9 ± 0.2	1.0 ± 0.2	0.10
CKD-EPI (mL/min/1.73m^2)	85 ± 13	79 ± 13	0.03
Urinary protein excretion (g/24h)	0.1 [0.0-0.3]	0.0 [0.0-0.2]	0.23
Two months post-donation			
Systolic bp (mmHg)	134 ± 14	129 ± 12	0.10
Diastolic bp (mmHg)	80 ± 9	81 ± 8	0.61
MAP (mmHg)	98 ± 10	97 ± 9	0.65
Serum creatinine (mg/dL)	1.4 ± 0.3	1.4 ± 0.3	0.56
CKD-EPI (mL/min/1.73m^2)	51 ± 11	49 ± 9	0.46
Urinary protein excretion (g/24h)	0.0 [0.0-0.2]	0.0 [0.0-0.2]	0.37
One year post-donation			
Systolic bp (mmHg)	134 ± 15	129 ± 15	0.10
Diastolic bp (mmHg)	84 ± 10	80 ± 8	0.08
MAP (mmHg)	101 ± 10	96 ± 8	0.05
Serum creatinine (mg/dL)	1.3 ± 0.2	1.3 ± 0.2	0.31
CKD-EPI (mL/min/1.73m^2)	58 ± 10	55 ± 11	0.22
Urinary protein excretion (g/24h)	0.1 [0.0-0.1]	0.1 [0.0-0.1]	0.64

Values represent mean ± SD; median [IQR] or n (%). bp: blood pressure; MAP: mean arterial pressure.

several donors stopped their antihypertensive medication, especially at one year post-donation. At five year post-donation, however, all but one hypertensive donors were using antihypertensive drugs again. Prior to and two months post-donation, none of the control donors used antihypertensive drugs. At one year post-donation, one control used a calcium channel blocker, while at five year post-donation four donors used one antihypertensive.

Pre- and post-donation characteristics for donors with one year follow-up are shown in table 3. Again, pre-donation systolic blood pressure and MAP were higher in hypertensive donors ($p<0.01$). This difference was again lost post-donation, though the difference in MAP reached statistical significance again at one year post-donation. Diastolic blood pressure was similar between hypertensive and control donors at all time-points. Figure 1b displays blood pressure course from two months post-donation to one year post-donation. In hypertensive donors, diastolic blood pressure showed a non-significant rise to one year post-donation,

control donors remained stable. Renal function at one year post-donation, expressed as serum creatinine and estimated GFR by CKD-EPI equation, was similar between the groups, as well as urinary protein excretion.

A small subset of donors had data on renal function and blood pressure five year post-donation. Characteristics are shown in table 1. From two months post-donation up to five year post-donation, blood pressure remained stable for hypertensive donors and controls (figure 1c). At five year post-donation, no difference in renal function or urinary protein excretion was observed. Figure 2b shows a parallel course in renal function to five year post-donation.

To evaluate the effect of blood pressure as such, correlation and regression analysis was performed. No associations were found between pre-donation GFR and blood pressure. Pre-donation systolic blood pressure correlated negatively with early post-donation GFR (R -0.22, $p<0.01$). This association was lost after correction for age. Neither diastolic blood pressure, nor MAP were significantly related to renal function. No significant associations were found between serum creatinine and CKD-EPI at one year and pre- and post-donation blood pressures. The group with five year follow-up was too small for continues analysis.

Discussion

In this study, we compared post-donation outcome of living kidney donors with pre-existent hypertension to matched control donors. Although hypertensive donors had higher blood pressure prior to and two months post-donation, these differences were lost at one and five year post-donation. At none of the evaluated time points differences were seen in renal function or urinary protein excretion between hypertensive and normotensive donors. Where normotensive donors showed a rise in diastolic blood pressure, hypertensive donors retained stable blood pressure throughout the follow-up. Thus, post-donation course of renal function and blood pressure of hypertensive donors is comparable to normotensive donors, including the long term adaptive increase of GFR between year one and five following donor nephrectomy.

Earlier studies evaluating the post-donation follow-up of living kidney donors had some conflicting results. While several studies reported an increase in blood pressure post-donation of 5-10 mmHg (2-5,11) others found no increase (12,13). The latter studies, however, had rather short follow-up (maximum one year) which may explain the lack in increase in blood pressure. The incidence rate of post-donation hypertension varies from 15 % (14), to 22% (15) and 45% (16). This incidence appears to increase with increasing time post-donation, and indeed the highest reported incidence was after a mean follow-up of fourteen years (16). Although hypertension is, thus, present post-donation, incidence rates are similar to the general population when correcting for age and gender (3,17-19). One study, however, found a higher incidence of hypertension in male donors compared to matched controls. Donors with post-donation hypertension had higher macro albuminuria and urinary protein excretion than normotensive donors in three studies (5,15,17). Here, however, we found no difference in urinary protein excretion. Textor et al. as well compared donors with pre-existent hypertension to normotensive donors, and found no increase in blood pressure or differences

in renal function or urinary protein excretion (6). However, the definition of hypertension was made on blood pressure cut-offs, and only three patients used antihypertensive drugs pre-donation. One study reported that donors with post-donation hypertension are at increased risk for an estimated GFR <60 ml/min/1.73m2 (20). In this study, however, we found no difference in renal function in the short and long term post-donation.

The finding that hypertensive donors are not at increased risk for renal function impairment post-donation is reassuring for the current donor selection practice. Due to a shortage in donor organs, many centers accept more marginal donors nowadays. Although this study shows no deleterious effects of pre-donation hypertension, we like to emphasize that donors in our center undergo strict selection, comprising renal function measurements and strict blood pressure management. Where one might expect lower renal function in middle-aged hypertensive subjects, our donors actually had very good renal function pre-donation. This contradiction can be explained by the selection bias due to the screening. Thus, we stress the need for thorough donor screening before accepting donors with hypertension. Furthermore, post-donation follow-up of living kidney donors remains important, and enables identification of subjects at risk for renal function loss or hypertensive complications.

Previous studies have shown the importance of ambulatory blood pressure measurements as part of the donor screening (21-23). Unfortunately, ambulatory measurements were not available for all donors of this study. Prior to, two months post-donation and five year post-donation, blood pressure was measured during renal function measurement. Donors were seated in a quiet environment in semi-supine positions, and were at rest for at least two hours before the blood pressure measurement started. Blood pressure was recorded for at least halve an hour. At one year post-donation, only office blood pressures were available.

Our study has several limitations, the most important being the relatively small sample-size, the mono centric character, and the lack of standardized ambulatory blood pressure measurements. Furthermore, the conclusions cannot be extrapolated to patients with African ethnicity.

In conclusion, hypertensive living kidney donors show a similar course in post-donation renal function and blood pressure as normotensive donors. Thus, hypertensive donors are not at increased risk of renal function loss up to five year post-donation. More long-term studies, with longer follow-up of donors with hypertension prior to donation, are necessary to ensure long term donor safety.

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Chapter 5

Five year follow-up of living kidney donors: effect of donor risk profile on kidney outcome

5

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Abstract

Background: To increase the living donor pool, selection criteria have become less strict, enabling acceptation of older, overweight, hypertensive and smoking donors. This study evaluates the effect of the donor risk profile on donor renal outcome five years post-donation.

Methods: Evaluated were 133 living kidney donors. Kidney function (GFR, ^{125}I-iothalamate) was measured at 6±7 months prior to and 2±0 months and 5.4±1.5 years after donation.

Results: Mean GFR at five years was 82±15 mL/min. At donation, 25% of donors was aged>55 years, 17% was obese, and 20% used antihypertensive drugs or had a blood pressure> 145/85 mmHg. Donor age associated negatively with five years GFR (R-0.27, p<0.01). Donors older than 55 years had lower GFR at all time-points, but kidney function course was similar to that of younger donors. Donor BMI, hypertension or smoking behavior showed no influence on post-donation kidney function course. In multivariate analysis, pre- and early post-donation GFR were the strongest predictors of five years GFR (R^2 0.65 and 0.69), followed by age and gender.

Conclusion: Overall five years outcome is good. When pre-donation kidney function is assured to be good, the current practice of accepting older, overweight, hypertensive and smoking donors is safe up to five years post-donation.

Introduction

During the last decade, living kidney donors have become of major importance for transplant programs, supplying up to half of the donor organs (1-3). Many studies reported excellent donor outcome on short- and long term post-donation (4-9). These donors, however, were strictly screened, and represent a very healthy part of the population. To increase the donor pool, donor selection criteria have become less strict (10;11). Nowadays, older subjects, subjects with overweight or obesity, and even hypertensive subjects may be accepted for living kidney donation. These traits are known risk factors for the development of kidney disease. Higher age and body mass index (BMI) were previously shown to results in lower early post-donation kidney function (12-15), though others showed no effect. Nevertheless, the altered donor characteristics warrant close monitoring of long term donor safety, in particular regarding kidney function.

In this study, therefore, we evaluate the effect of the donor risk profile, i.e. donor age, BMI, blood pressure and smoking behavior, on donor outcome five year post-donation.

Methods

In this study, 133 living kidney donors were evaluated. All donated between 1984 and 2006 in the University Medical Center Groningen. Kidney function measurements were performed as part of the routine donor screening and early follow-up program at 6±7 months prior to and 2±0 months after donation. Since 2007, all former donors in our centre are invited for a second post-donation kidney function measurement, approximately five years after nephrectomy. Donors who donated before 2002 (n=93) were all invited in one follow-up round, several off these donors already had a second follow-up on their own request. Of the 262 invited donors, 170 (65%) responded for follow-up. For inclusion in the current analysis, we selected all donors for whom complete data on all three time points were available, 37 donors were excluded. At long term follow-up, mean duration since donation was 5.4 ± 1.5 year, with a range of 2.0 to 11.6 years. Procedures were conducted in accordance with the Helsinki declaration.

Routine donor screening

All potential donors at our center undergo gold standard kidney function measurement, as described below. The basal kidney function measurement is extended by two hours in which the kidney function is stimulated by dopamine, to measure the so called reserve capacity. Donors were eligible to donate with a glomerular filtration rate (GFR) > 80 mL/min. For older subjects or small females, an exception could be made based on a good response to dopamine infusion, with a stimulated GFR > 80 mL/min. Furthermore, the response in dopamine is judged, especially when GFR is close to 80 mL/min. Since 2002, donors who use antihypertensive drugs are allowed to donate. A maximum of two antihypertensive drugs is tolerated, provided ambulatory blood pressure does not exceed 150/85 mmHg. Donors with a body mass index (BMI) exceeding 30 kg/m^2 are encouraged to lose weight. There is no maximum for age, but many older potential donors are excluded due to low GFR or comorbidities. Potential donors with a disturbed glucose tolerance test are rejected, as well

as donors with severe atherosclerotic lesions. Mainly for anatomical concerns, an abdominal CT scan is performed for all donors. Smoking behavior has never been a selection criterion at our center.

Kidney function measurements during screening and follow-up

GFR was measured by constant low-dose infusion of the radio-labelled tracer ^{125}I iothalamate as described previously (44-46). Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. Filtration fraction (FF) was calculated as GFR/ERPF. For the measurements, subjects were seated in a quiet room in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period followed, after which the clearance periods started. Clearances were measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%. Next to basal kidney function, kidney reserve capacity was measured pre- and two months post-donation as part of screening and early follow-up. To obtain reserve capacity, the above-mentioned procedure was extended for two hours. During this period, dopamine was infused at a rate of 1.5 µg/kg per minute.

Calculations and classification

Mean arterial pressure (MAP) was calculated as: MAP = (1/3 [systolic pressure - diastolic pressure] + diastolic pressure). The change in GFR and ERPF due to stimulation by dopamine was calculated as (stimulated kidney function – basal kidney function), giving ΔGFR_{DOPA} and $\Delta ERPF_{DOPA}$. Since donor selection, surgical techniques and follow-up procedures have changed over time, we wanted to evaluate the effect of time of donation on donor characteristics and five year GFR. Therefore, a continuous variable was developed by calculating the duration between donation and an artificial date: January 1st, 2007; in years. Thus, a donor who donated at September 12, 2006 has a value of 0.3 years and a donor who donated at November 28, 2000 has a value of 6.1 years. Cut-offs for high risk donors were pre-donation age ≥ 55 years, BMI > 30 kg/m^2 (47), current smoking of the donor, and antihypertensive drug use or a donor blood pressure > 145/85 mmHg.

Statistical analysis

Analyses were performed using PASW Statistics version 18 and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [IQR]. Independent samples *t* Test, Mann-Whitney test and chi-square test were used to analyse for differences between groups. Linear regression was used for prediction of five year GFR. For gender, male gender was set as reference category.

Results

Donor characteristics pre- and both early and five years post-donation are shown in table 1. On average, this donor population is approximately 50 years old, slightly overweight and normotensive. Early post-donation GFR fell to 64 ± 6 % of the pre-donation value. Five years post-donation, GFR had increased to 71 ± 8 % of the pre-donation value. ERPF remained stable, at 67 ± 7 % early post-donation, and 66 ± 10 % five year post-donation.

Selection habits of our center have changed over time, causing a change in donor characteristics, as displayed in figure 1. Over time, our center accepted more donors aged over 55 years, with overweight or obesity and/or with antihypertensive drugs use or a blood pressure > 145/85. Donor age and BMI at time of donation both related negatively with time since the date of donation (calculated as duration between donation and January 1st 2007; R -0.19 and -0.24, both p<0.01), indicating that the most recent donors were older and more overweight than donors who donated longer ago. Table 2 shows the donor risk profile of the whole cohort prior to donation. Seventeen percent of donors was obese, and 20% used antihypertensive drugs or had a blood pressure > 145/85 mmHg.

Influence of donor risk factors on post-donation kidney function

Univariate associations between pre-donation risk factors age, BMI and systolic blood pressure with GFR at five years after donation are shown in figure 2. The strongest association with GFR at five years was provided by pre-donation GFR (R2 0.65, p<0.01). Age at donation was negatively associated to five year GFR (R^2 0.25, p<0.01), while BMI and blood pressure showed no association. Smoking behavior showed no influence on five year post-donation GFR.

Table 1: Donor characteristics prior to, and early and five year post-donation.

	Total	Male	Female
Duration of follow-up (years)	-0.5 ± 0.6	0.2 ± 0.0	5.4 ± 1.5
Age (years)	48 ± 11	49 ± 11	54 ± 11
BMI (kg/m^2)	26 ± 4	26 ± 4	27 ± 4
Systolic blood pressure (mmHg)	124 ± 12	125 ± 13	126 ± 12
Diastolic blood pressure (mmHg)	75 ± 9	76 ± 8	77 ± 8
Serum creatinine (mg/dL)	0.9 ± 0.2	1.3 ± 0.3	1.2 ± 0.2
GFR (mL/min)	116 ± 20	74 ± 13	82 ± 15
ERPF (mL/min)	438 ± 80	291 ± 52	284 ± 58
FF (%)	27 ± 3	26 ± 3	29 ± 4
GFR_{DOPA} (mLmin)	13 ± 11	3 ± 5	-
$ERPF_{DOPA}$ (mLmin)	118 ± 54	50 ± 30	-

Values represent mean ± SD. ΔGFR_{DOPA}: change in GFR by stimulation with dopamine: (stimulated GFR – basal GFR); $\Delta ERPF_{DOPA}$: change in ERPF by stimulation with dopamine: (stimulated ERPF – basal ERPF).

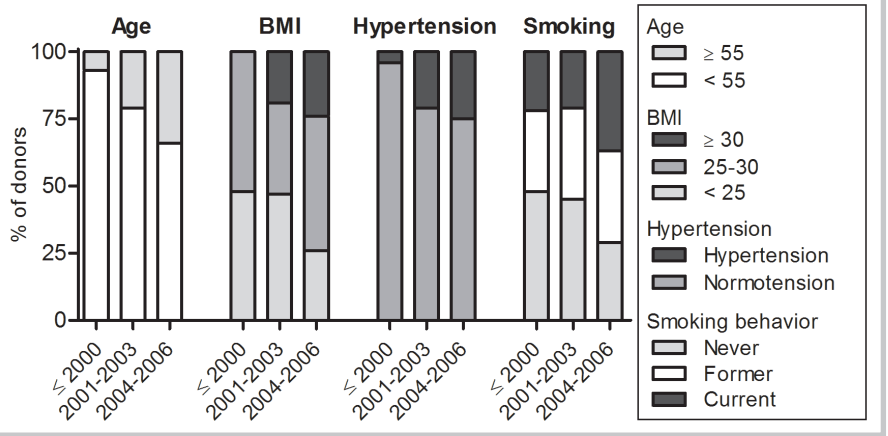

Figure 1: Change in donor characteristics over time. Bars represent percentage of donors by break-up in age, BMI, hypertension and smoking behavior. Numbers per group are: ≤ 2000 n= 27; 2001-2003 n= 38; and 2004-2006 n=68.

Table 1: Donor characteristics prior to, and early and five year post-donation.

	N (%)	*% Male*	Age (years)	BMI (kg/m²)	GFR (mL/min)	ΔGFR_{DOPA} (mL/min)
Age (years)						
<55	100 (75)	41	47 [40-52]	26 [23-28]	118 [104-129]	14 [8-19]
>55	33 (25)	48	60 [57-63][a]	27 [25-29]	105 [90-121] [a]	9 [1-16] [a]
BMI (kg/m²)						
<25	49 (37)	39	48 [43-54]	23 [22-24]	107 [102-122]	14 [7-19]
25-30	61 (46)	46	51 [43-55]	27 [26-28][a]	118 [103-130]	13 [7-18]
≥30	23 (17)	43	52 [43-55]	32 [31-33][a]	121 [108-140]	13 [1-19]
Hypertension						
Normotensive	107 (80)	41	49 [42-55]	26 [24-28]	116 [102-127]	13 [7-18]
Hypertensive	26 (20)	50	53 [49-59]*	28 [25-30]*	122 [110-128]	15 [7-19]
Smoking						
Never	50 (38)	44	51 [44-58]	26 [22-28]	112 [100-127]	11 [6-18]
Former	39 (29)	41	51 [45-56]	27 [25-28]	117 [101-130]	14 [5-18]
Current	44 (33)	43	48 [41-54]	26 [24-29]	118 [104-128]	13 [8-20]

Values represent N (%) and median [IQR]. [a] difference by default; *p<0.05 vs. corresponding value. ΔGFRDOPA: change in GFR by stimulation with dopamine: (stimulated GFR – basal GFR)

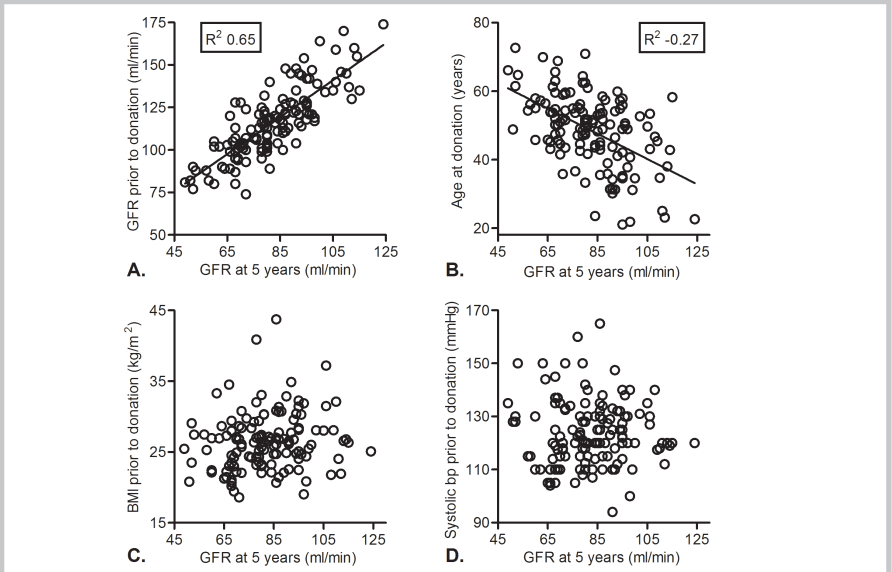

Figure 2: scatter plots for five year donor GFR with A) pre-donation GFR, B) age at donation; C) pre-donation BMI); and D) pre-donation systolic blood pressure. R^2 represents adjusted R^2 by linear regression analysis.

5

Figure 3 shows the time course of post-donation kidney function by a break-up in donor risk factors. Older donors had lower kidney function at all time points (all p<0.01), but the adaption to five years was similar to that of younger donors. Donors with a BMI >25 kg/m^2 had higher GFR early post-donation than normal weight donors, but this difference was lost after correction for body surface area (data not shown). Normotensive donors and

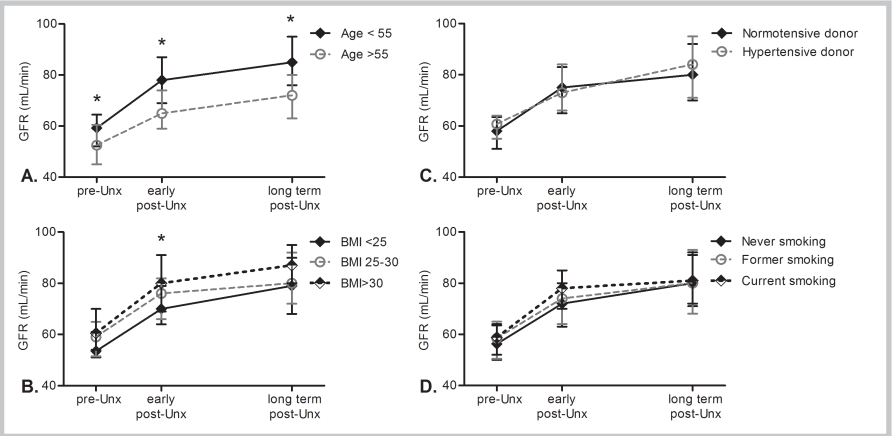

Figure 3: Change in GFR by break-up in A) pre-donation age; B) pre-donation BMI; C) pre-donation hypertensive status; D) pre-donation smoking behavior. Values represent median [IQR]. * p<0.05 *vs*. corresponding lowest category . Hypertension was defined as use of antihypertensive drugs, or a blood pressure >145/85 mmHg prior to donation. GFR prior to donation (pre-Unx) is shown as 'single kidney GFR': (basal GFR / 2).

hypertensive donors had a similar course in kidney function, as had donors with different smoking habits.

Prediction of five year donor outcome

Five year GFR was 73 ± 12 mL/min, with a range of 50 to 124 mL/min. Of pre-donation characteristics, pre-donation GFR associated the strongest with five years GFR (adjusted [a] R^2 0.65, standardized [std.] β 0.81; p<0.01; figure 1). On univariate analysis, other predictors of five year GFR were GFR_{DOPA} (aR^2 0.61, std. β 0.78; p<0.01); pre-donation age (aR^2 0.26, std. β-0.52; p<0.01) and gender (aR^2 0.13, std. β -0.37 [reference male gender]; p<0.01). Pre-donation BMI, blood pressure and smoking behavior did not associate significantly to five year GFR. The time of donation also did not affect five year outcome. On multivariate analysis, five year GFR was best predicted by the model with pre-donation GFR and age (aR^2 0.69, p<0.01; std. β 0.71 and -0.23, both p<0.01). Due to co-linearity with crude GFR, GFR_{DOPA} was removed from the model.

Early post-donation characteristics had their influence on five year GFR as well. Again, the strongest association was provided by early post-donation GFR (aR^2 0.69, std. β 0.83 ; p<0.01). Early post-donation GFR_{DOPA} associated with five year GFR as well (aR^2 0.63, std. β 0.80 ; p<0.01). There were no associations with early post-donation BMI or blood pressure. In combination with the pre-donation characteristics, five year GFR was predicted by a model with pre- and early post-donation GFR, age and gender (aR^2 0.75, p<0.01; std. β 0.32, 0.46, -0.15 and -0.11, all p<0.05). Again, GFR_{DOPA} was removed due to co-linearity with crude GFR.

Discussion

This study evaluated the effect of the donor risk profile on donor outcome five year post-donation. As expected, donor age and pre-donation kidney function were the most important modulators of post-donation kidney function. Other risk factors, BMI, blood pressure and smoking behavior, did not influence donor outcome. Overall donor outcome was good; all donors had sufficient kidney function at five year post-donation, without development of proteinuria.

This is the first study that evaluates the effect of the total donor risk profile on five years post-donation outcome with gold standard kidney function measurements. Previous studies mainly focused on one or two risk factors, mainly age and BMI. Below, we will discuss the separate effects of the donor risk factors first.

Kidney function is known to decline with age (16;17), mean reported decline is about 0.75 mL/min per year, though rates of decline may differ (18). This translates in lower pre- and post-donation kidney function in older donors (14). Post-donation kidney function course, however, is similar between older and younger donors (19;20). This is in line with our findings. A previous study from our center found that while pre-donation reserve capacity is similar between older and younger donors, early post-donation reserve capacity is lower in older donors (15). Apparently, this does not disturb the adaptive response to five years post-donation.

Obesity is a known risk factor for the development of kidney disease in the general

population (21-24), and especially after donor nephrectomy (12;13;15). Obesity was linked to glomerulomegaly and glomerulosclerosis in very severe cases (25-27). In this study, we found no detrimental effects of overweight and obesity. In the Netherlands, however, the current mean body mass index is about 26 kg/m^2, and 17% of our donors had a BMI > 30 kg/m^2. Though this is almost one fifth of the population, and the prevalence is still growining, the prevalence may be lower than in other western countries. Nevertheless, our findings are in line with previous studies focusing on the effects of overweight on donor outcome (28;29).

Since 2002, our center accepts potential donors with pre-existent hypertension. The actual number of donors that donated that were accepted despite the use of antihypertensive drugs, however, is still low. Hypertension is an important risk factor for kidney damage and kidney function loss in the general population (30-32). In kidney donors, so far one study focused on the effect of pre-existent hypertension on donor outcome (33). Fortunately, no detrimental effects were found. While several studies reported a rise in blood pressure post-donation (14;34), blood pressure remained stable in this study.

Smoking is an important risk factor for the development of cardiovascular disease and cancer. Previous studies showed that smoking is also a risk factor for kidney damage, because of both vascular and direct endothelial damage (35-37). Smoking may accelerate the aging-related decline kidney function (38) and increase albuminuria in hypertensive patients (36). Here, however, we observed no difference in kidney function between current smokers and current non-smokers, neither on short nor on five year follow-up. Thus, smoking behavior does not appear to influence post-donation kidney function course. At our center, smoking behavior is not a criterion in kidney donor selection. Although donors may be advised to stop smoking, there is no further special attention for smoking behavior in screening or follow-up.

Where many studies focus on one or two potential risk factors, for the individual donor it is the whole risk profile that counts. When we combined all risk factors in regression analysis, pre-donation GFR and donor age were the only significant factors for prediction of five year kidney function. This is in line with abovementioned discussion. Thus, as long as kidney function is at a sufficient level prior to donation, the current practice of accepting older, more overweight and even hypertensive donors appears to be safe. Here we like to note, however, that the donors described here all had gold standard kidney function measurements as part of the screening program. Since a cutoff of 80 ml/min was applied, all donors had excellent kidney function prior to donation, including donors with an unfavorable risk profile. Furthermore, the measurement of renal reserve capacity by use of dopamine ascertained adaptive capacity, even when GFR was near 80 mL/min. Although this is an important strength of screening with the use of gold standard kidney function measurements, it may hamper generalizability to other populations in which an estimation of kidney function is used. Since the pitfalls of screening with estimating equations are well known (39-43) – 59% of donors presented here have an estimated GFR by MDRD Study equation < 80mL/min/1.73m^2 (data not shown) –, we like to emphasize the importance of thorough kidney function measurement prior to donation.

Other limitations of this study are the rather short duration of follow-up and the moderate risk profile of our donors. Although our center does not use an absolute cutoff for donor age, only 14 donors were aged above 60 years. As mentioned earlier, only 17% of

donors had a BMI > 30kg/m^2.

In summary, overall five year donor outcome is good. Although older donors have lower kidney function post-donation, adaption to five year post-donation is similar to younger donors. When kidney function prior to donation is assured to be good, the current practice of accepting older, overweight, hypertensive and smoking donors is safe up to five years post-donation.

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Chapter 6

Former living kidney donors are not CKD patients post-donation: effect of CKD classification

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Abstract

Background: many living kidney donors have an estimated kidney function <60 mL/min/1.73m^2 post-donation, and, thus, meet to the criteria of CKD stage 3. However, the prognostic impact of a given GFR in two diseased kidneys may not be equivalent to the same GFR in one healthy kidney. To test this assumption, we compared kidney function course of former donors to age, gender and baseline GFR matched CKD patients.

Methods: included were 57 kidney donors, with baseline values obtained 2 months post-donation, and 57 CKD patients. All had repeated GFR (^{125}I-iothalamate) and ERPF (^{131}I-hippuran) measurements after 4.7±1.5 years.

Results: at baseline, 25% of donors met with criteria for CKD stage 3. In donors, GFR increased over time despite a slight fall in ERPF, while GFR and ERPF both fell in CKD (both p<0.01 vs. donors). CKD stage improved to stage 2 or less in 7/14 donors with stage 3 at baseline, whereas it worsened in 15 CKD patients.

Conclusion: kidney function course differs substantially in kidney donors and CKD patients. Although many donors meet criteria of CKD stage 3, their prognosis diverges from true CKD and they should not be regarded as CKD patients. CKD classification is not applicable in former kidney donors.

Introduction

Living kidney donors are of great importance in kidney transplantation. Donor nephrectomy is known to be a safe procedure, and studies reporting donor outcome all show reassuring results (1-6). Many former kidney donors, however, have an estimated kidney function <60 mL/min post-donation, and, thus, meet the criteria of chronic kidney disease (CKD) stage 3.

CKD staging is based on prognostic impact of kidney function and markers of kidney damage and/or pathological abnormalities (7). For kidney function, estimated glomerular filtration rate (eGFR) is most often used, whereas additional incorporation of albuminuria in the classification has been claimed to improve its prognostic impact. For former kidney donors, the use of CKD stages based of eGFR, however, raises two problems. First, the prognostic impact of the CKD stages is based on the presence of two kidneys. The prognostic impact of a given GFR in two diseased kidneys, however, may not be equivalent to the same GFR in one healthy kidney. Furthermore, it is known that eGFR tends to underestimate true kidney function, especially in healthy subjects (8-12). Based on the literature, it can be anticipated that the prognosis for a given eGFR, and hence CKD classification, is much more favorable in former kidney donors. Barri et al. showed that a substantial part of former donors has an GFR <60 mL/min/1.73m^2 at short term after donation, and that especially older donors are affected. Nevertheless, Ibrahim et al. showed in a large cross-sectional study that the risk of developing end stage renal disease is not increased in former donors, and that their health status at a mean of twelve year post-donation was comparable to that of matched controls. Taken together, use of CKD stages in former donors could lead to undue labeling of healthy kidney donors as 'patients', with possible adverse consequences, for instance in insurance matters.

Therefore, we evaluated the prognostic impact of CKD classes in former kidney donors determined two months post-donation, for predicting donor outcome at five year post-donation. Furthermore, we compared post-donation kidney function course to that of CKD patients, matched for gender, age and baseline kidney function.

Methods

In this study, 57 living kidney donors who donated at our centre between 1987 and 2006 were included. As part of the routine screening and follow-up, kidney function was measured four months prior and two months following donation. Since 2007, all former donors in our centre are invited for a second post-donation kidney function measurement five year post-donation. Donors who donated before 2002 were all invited in one follow-up round. Donors were selected for having complete data on the three time points, and on availability of a suitable match. Of the 202 donors that donated before 2007, 100 donors had complete data on all three time-points. There were no differences between donors that could be matched and donors whom could not be matched in terms of age, BMI, blood pressure and kidney function at baseline and end of follow-up. As controls, 57 non diabetic CKD patients were included. Donors and patients were matched for age, gender, baseline GFR and duration of follow-up. For the CKD patients, we retrieved data from all patients enrolled in renal hemodynamic studies performed between 1972 and 1995 at our center (13). Of the 77 patients with

complete data, 57 could be matched to a former donor. Of the CKD patients, 18 had essential hypertension; 10 membranous glomerulopathy; 8 focal glomerulosclerosis; 5 polycystic kidney disease; 4 IgA nephropathy; 3 had kidney disease of unknown origin; and 9 had other causes like Barrter syndrome and ischemic lesions. Procedures were conducted in accordance with the Helsinki declaration.

Measurement of kidney function

Glomerular filtration rate (GFR) was measured by constant low-dose infusion of the radio-labelled tracer ^{125}I iothalamate as described by Donker (14), Visser (15) and Apperloo et al. (16). Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. Filtration fraction (FF) was calculated as GFR/ERPF. For the measurements, subjects were seated in a quiet room in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%.

Calculations

In our centre, before 1st March 2006, creatinine was measured by Jaffé alkaline picrate assay, on the MEGA, Merck KGaA, Darmstadt, Germany. Thereafter, creatinine was measured by enzymatic assay on the Roche Modular. Both methods were calibrated to the reference standard, i.e. Cleveland Clinic Laboratory measurements, as described before (17). We used the CKD-EPI equation (18) for estimation of GFR (eGFR). CKD-EPI equation was calculated gender specific, and stratified by serum creatinine (SCr) levels. The following calculations were used:

Female with SCr ≤0.7 mg/dL: GFR = 144 * $(0.993)^{age}$ * $(SCr / 0.7)^{-0.329}$

Female with SCr > 0.7 mg/dL: GFR = 144 * $(0.993)^{age}$ * $(SCr / 0.7)^{-1.209}$

Male with SCr ≤ 0.9 mg/dL: GFR = 141 * $(0.993)^{age}$ * $(SCr / 0.9)^{-0.4111}$

Male with SCr > 0.9 mg/dL: GFR = 141 * $(0.993)^{age}$* $(SCr / 0.9)^{-1.209}$

No correction for ethnicity was applied, as none of the patients were of African ethnicity. Mean arterial pressure (MAP) was calculated as (1/3[systolic blood pressure – diastolic blood pressure] + diastolic blood pressure). Body surface area (BSA) was calculated as according to DuBois (19). GFR and ERPF were normalized by dividing the raw sample by BSA and multiplying it with 1.73, giving GFR/BSA and ERPF/BSA. The slope of kidney function loss was calculated as (last value – baseline value)/duration of follow-up; giving a slope in mL/min/1.73m^2 per year.

Statistical analysis

Analyses were performed using PASW Statistics version 18 and GraphPad Prism version 5. Data are given as mean ± standard deviation or median [IQR]. Independent samples *t* Test, Mann-Whitney test and chi-square test were used to analyze for differences between groups. Differences within groups were tested with paired samples *t*-test and Wilcoxon Signed Ranks Test. We fitted linear regression models using a backward selection method to evaluate influence of baseline characteristics on donor outcome: GFR/BSA, ERPF/BSA and MAP at end of follow-up. Covariates were age, gender and baseline BMI, MAP, GFR/BSA and ERPF/BSA.

Results

Characteristics at baseline and end of follow-up for donors and CKD patients are displayed in table 1. As donors and CKD patients were matched for gender, in both groups 63% (n= 36) was male. Baseline age, glomerular filtration rate (GFR) and duration of follow-up were also similar between the groups, reflecting a good match. Compared to the CKD patients, living donors had higher body mass index (BMI) and lower blood pressure, urinary protein excretion (UPE) and effective renal plasma flow (ERPF) adjusted for body surface area (BSA) at baseline. At end of follow-up, donors had lower blood pressure, lower serum creatinine and UPE, and higher GFR/BSA and CKD-EPI values. Since long term ERPF was similar between donors and CKD patients, donors had a higher filtration fraction (FF) at end of follow-up. At baseline, 7 donors used antihypertensive drugs compared to 51 CKD patients. Most subjects used one drug, 4 donors and 25 CKD patients used and angiotensin converting enzyme inhibitor or angiotensin receptor blocker. The use of antihypertensive drugs increased to 11 donors and all 57 CKD patients at end of follow-up. Early post-donation, 14 donors had a GFR/BSA <60, and 35 a CKD-EPI <60 mL/min/1.73m^2. Long term post-donation 7 donors had a GFR/BSA <60, and 30 a CKD-EPI <60 mL/min/1.73m^2. Of the CKD patients, 11 had died up to 2011. Mean age at death was 65 ± 10 years, time since end of follow-up was 10 ± 4 years, none had died during follow-up. Up to 2011, none of the former donors had died.

Change in kidney function over time was significantly different between donors and CKD-patients (table 2 and figure 1). Where donors showed a negative serum creatinine slope and positive GFR/BSA and CKD-EPI slope, CKD patients showed a decrease in kidney function over

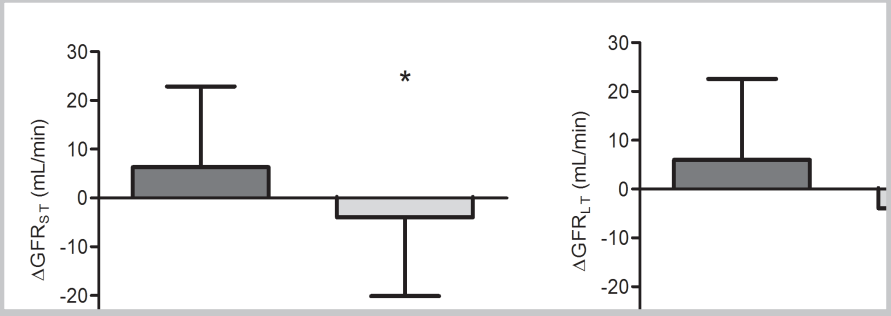

Figure 1: kidney function course for living kidney donors and CKD patients. Graphs represent GFR/BSA (top graph), CKD-EPI and ERPF/BSA (bottom graph). Donors show a positive kidney function course for GFR/BSA and CKD-EPI, and less steep negative course for ERPF/BSA than CKD patients. Symbols represent mean ± SD.

Table 1: donor and patient characteristics at baseline and end of follow-up.

	Donors	CKD patients	*P* value
Baseline			
Age (years)	48 ± 12	48 ± 11	1.00
BMI (kg/m^2)	27 ± 4	25 ± 3	0.02
Systolic blood pressure (mmHg)	126 ± 13	137 ± 18	<0.01
Diastolic blood pressure (mmHg)	77 ± 9	83 ± 12	<0.01
Serum creatinine (mg/dL)	1.4 ± 0.2	1.3 ± 0.4	0.57
UPE (g/24h)	0.0 [0.0-0.1]	0.7 [0.0-2.9]	<0.01
GFR/BSA (mL/min/1.73m^2)	67 ± 11	71 ± 18	0.21
CKD-EPI (mL/min/1.73m^2)	56 ± 11	63 ± 22	0.08
ERPF/BSA (mL/min/1.73m^2)	264 ± 48	304 ± 128	0.03
FF (%)	26 ± 3	25 ± 6	0.34
Subjects on antihypertensive drugs (n [%])	7 (12)	51 (89)	<0.01
End of follow-up			
Duration of follow-up (years)	4.9 ± 0.9	4.6 ± 1.9	0.27
Systolic blood pressure (mmHg)	124 ± 12	144 ± 31	<0.01
Diastolic blood pressure (mmHg)	77 ± 9	86 ± 14	<0.01
Serum creatinine (mg/dL)	1.2 ± 0.2	1.5 ± 0.5	<0.01
UPE (g/24h)	0.0 [0.0-0.2]	1.0 [0.0-2.5]	<0.01
GFR/BSA (mL/min/1.73m^2)	73 ± 12	63 ± 21	<0.01
CKD-EPI (mL/min/1.73m^2)	63 ± 13	54 ± 20	<0.01
ERPF/BSA (mL/min/1.73m^2)	247 ± 48	258 ± 95	0.44
FF (%)	30 ± 4	25 ± 7	<0.01
Subjects on antihypertensive drugs (n [%])	11 (19)	57 (100)	<0.01

Values represent mean ± SD and median [IQR]. In both groups, 63% of subjects was male. All subjects completed follow-up. UPE: urinary protein excretion; BSA: body surface area; FF: filtration fraction.

time. Though ERPF/BSA decreased in both donors and CKD patients, the decrease in donors was less steep. For 22 donors, intermittent creatinine values were available between baseline and end of follow-up. Of these 22 donors, 16 showed a gradual decrease in creatinine between baseline and end of follow-up, while 6 showed a decrease between baseline and one year post-donation and had stable creatinine values (± 0.05 mg/dL) up to end of follow-up.

Table 2: slopes of serum creatinine, GFR/BSA, CKD-EPI equation and ERPF/BSA.

	Donors	CKD patients	*P* value
Creatinine (mg/dL/year)	-0.03 ± 0.03	0.03 ± 0.07	<0.01
GFR/BSA (mL/min/1.73m2/ year)	1.8 ± 1.6	-1.4 ± 3.4	<0.01
CKD-EPI (mL/min/1.73m2/ year)	1.2 ± 1.6	-1.7 ± 2.8	<0.01
ERPF/BSA (mL/min/1.73m2/ year)	-3.2 ± 6.5	-9.8 ± 15.7	<0.01

Values represent mean ± SD. BSA: body surface area.

Effects of classification

Classification of kidney function according to the K/DOQI guidelines is displayed in figure 2. The top panel shows classification based on kidney function and UPE; the lower panel is solely based on kidney function. The top figure shows that where donors tend to move up in stage over time, CKD patients show progression. Furthermore, classification with CKD-EPI leads to more subjects with stage 3 CKD compared to classification with GFR in both groups. Since only very few donors have UPE > 0.5 g/day, CKD stage 2 is sparse in former donors. When classification is solely based on kidney function (lower panel), the percentage of kidney donors and CKD patients with stage 2 GFR or CKD-EPI shows a major increase, though the further pattern is similar to the top panel.

Implication of classification is displayed in figure 3. Kidney function course is shown for donors and CKD patients separately, by break-up in baseline kidney function. Subjects are not matched for this analysis; to obtain more equal group sizes, crude GFR was used instead of GFR/BSA, when GFR/BSA was used a similar pattern was seen (data not shown). The figure shows that donors show an opposite course in kidney function than CKD patients (p< 0.05

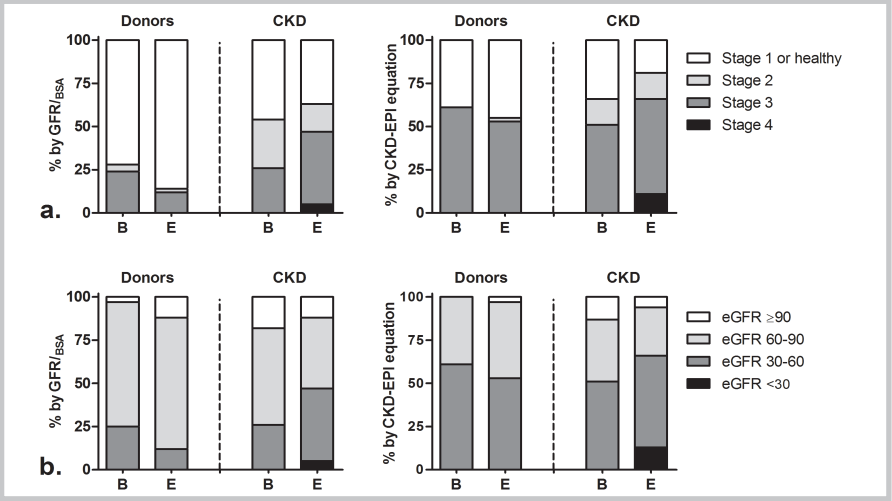

Figure 2: CKD stages by GFR/BSA (left graphs) and CKD-EPI equation. For figure a CKD stages were defined as: 1) (e)GFR >90 and UPE>0.5 g/day; 2) (e)GFR 60-90 and UPE>0.5 g/day; 3) (e)GFR 30-60; 4) (e)GFR<30. For figure b CKD stages were defined solely by kidney function. B: baseline; E: end of follow-up.

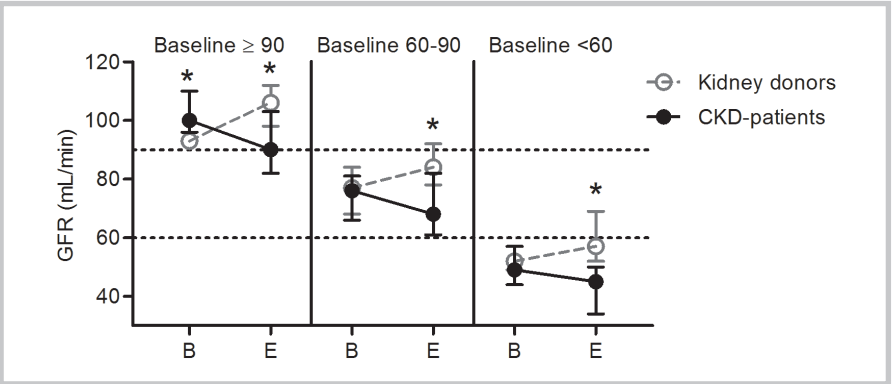

Figure 3: kidney function course from baseline (B) to end of follow-up (E) for donors and CKD patients, with a break up for baseline kidney function. Subjects were not matched. Group sizes are: n=11, 41 and 5 for donors and n=17, 30 and 10 for CKD patients. Symbols represent median [IQR] values. * p<0.05 vs. CKD patients. The kidney function slopes of kidney donors were significantly different than those of the CKD patients.

for change in kidney function), independent of baseline kidney function. When the break-up was made for CKD classification with proteinuria, a similar pattern was seen (data not shown).

Prediction of donor outcome

GFR/BSA at end of follow-up was best predicted by baseline GFR/BSA (adjusted R^2 0.71, standardized (std.) beta 0.85, p<0.01). Addition of baseline ERPF/BSA (std. beta 0.40) increased the R^2 to 0.77. Other donor characteristics had no significant influence on donor outcome. ERPF /BSA at end of follow-up was best predicted by baseline ERPF/BSA (adjusted R^2 0.68, std. beta 0.83, p<0.01). None of the other donor characteristics added significantly to the model. MAP at end of follow-up was best predicted by MAP at baseline (adjusted R^2 0.43, std. beta 0.67, p<0.01), without influence of other characteristics.

Discussion

This study compares kidney function course of former living kidney donors to that of matched CKD patients. Whilst CKD patients show a decline in both GFR and ERPF over time, former donors show an increase in GFR between two months and five year post-donation, and a less steep decline in ERPF compared to the CKD patients. Prognostic impact of early post-donation CKD staging for prediction kidney function impairment in former donors is low. Thus, living kidney donors are not CKD patients post-donation.

Several studies evaluating long term donor outcome reported excellent long term results (1;3-6). One study even showed that post-donation kidney function can keep increasing up to fifteen year post-donation (2). In this study GFR increased to long term, but ERPF showed a slight decrease. This different course increases the filtration pressure in the kidney, expressed by an increase in FF of 25% to 30%. At five year post-donation, however, we find no detrimental effects of this higher filtration pressure. Although it has been suggested that post-donation kidney function increases up to one year post-donation and then slowly

declines, here we find improvement or stabilization of kidney function between one and five year post-donation in a subset of donors.

Although many studies report excellent long term results, many kidney donors have an eGFR<60 mL/min/1.73m^2 post-donation (12,20-25). This marks former donors as CKD patients, with possible negative socio-economic consequences in some countries. The current CKD staging, as proposed by the KDIGO (7), is based on the prognostic impact of a given GFR in two kidneys. A low GFR in a subject with two kidneys may reflect kidney disease, while the same GFR in a former donor reflects a perfectly healthy single kidney. Other staging systems have been proposed (23-25), based more strongly on the actual presence of kidney damage. In this study, classification based on proteinuria and kidney function classifies nearly all former donors with a kidney function > 60 mL/min/1.73m^2 as healthy, whereas classification solely on base of kidney function shows more variation. Furthermore, use of estimated GFR may lead to a far worse pattern in classification than with measured GFR. Although CKD-EPI performs better than MDRD study equation in the range > 60 mL/min/1.73m^2 (26), it still underestimates measured kidney function. In this study, use of CKD-EPI for classification led to a higher CKD stages in both former donors and CKD patients. As many centers rely on eGFR rather than gold standard kidney function measurements, the prevalence of CKD may be overestimated.

Our study has several limitations, the most important being the small sample size and rather short duration of follow-up. Furthermore, our donors were screened by use of gold standard kidney function measurement prior to donation, which makes them highly selected for good kidney function. This may hamper comparison to donor populations screened by eGFR. Due to the shortage in donor organs, selection criteria for potential donors have become more liberal over the last decade, with acceptation of older, overweight and even hypertensive donors (Mandelbrot). Of the donors described in this study, 7 (12%) were aged above 60 years, 10 (18%) had a BMI exceeding 30 kg/m^2 and 7 (12%) used antihypertensive drugs prior to donation. Current donor populations may have less favorable risk profiles, which may influence long term outcome. Since kidney function was only measured twice post-donation, slope analysis in this study was based on only two time points. Since the first time point was early post-donation, the compensatory increase in kidney function was probably not complete at the time of measurement, which may have biased the slope assessment. In a subset of donors in whom creatinine measurements at more time points were available, creatinine decreased up to one year post-donation, and kept decreasing or stabilized up to five year post-donation. Thus, the use of this early time point may have led to a more positive slope in former donors, but based on our subgroup analysis this cannot explain the large difference in slope between former donors and CKD patients. Since all donors at our center were Caucasian, the conclusions may not be representative for donors with other ethnicities.

What are the implications of this study? First, we like to emphasize that former living kidney donors are healthy, and even with a low kidney function should not be regarded as patients. As shown here, for a given GFR the course in kidney function is very different for donors and true CKD patients. Thus, current CKD staging for prognostic purposes is not applicable in former kidney donors. Nevertheless, it should be pointed out that CKD staging has purposes other than estimating renal and overall prognosis, namely guiding safety

precautions related to the excretory capacity of the kidney for potentially toxic pharmacological and radiological agents. This related to clearance capacity per se, and for such purposes, actual kidney function should be taken into account.

In summary, for a given GFR former kidney donors have a substantially different course in kidney function than CKD patients. Even though many kidney donors may have an eGFR < 60 mL/min/1.73m^2 post-donation, they should not simply be regarded as CKD patients. To acknowledge the special nature of the former kidney donor, we suggest to consider former kidney donors a special category in future proposals for CKD classification .

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Chapter 7

Renal structural changes in living donor biopsies: associations with donor characteristics and short term renal outcome after donation

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Abstract

Background: Living donors are indispensible for kidney transplantation programs. More liberal donor criteria prompted us to evaluate the presence of early renal damage at time of donation and associations with donor characteristics and outcome.

Methods: We evaluated 82 living donors. All underwent GFR (^{125}I-iothalamate) and ERPF (^{131}I-hippurate) measurements four months prior and two months post-donation. Needle biopsies were taken during the donation procedure. Sections were stained for inflammation (neutrophillic granulocytes, macrophages), and pre-fibrosis (α-smooth muscle actin (α-SMA)). The degree of glomerulosclerosis, interstitial fibrosis, vascular hyalinosis and intima thickness were scored in PAS stained sections.

Results: The extent of interstitial α-SMA expression associated with donor age (R 0.32, $p<0.01$) and GFR and ERPF prior to donation (R -0.38 and -0.39, $p<0.01$) and early post-donation (R -0.36 and -0.41, $p<0.01$). The associations with kidney function were independent of donor age, the associations with post-donation kidney function were, however, dependent on pre-donation kidney function. No other associations with outcome were found.

Conclusion: Mild pre-existent renal damage is present in kidneys of living kidney donors at the time of donation. These changes, however, show no influence on adaptive capacity. Early adaptive capacity following kidney donation is preserved in the presence of mild pre-fibrotic changes.

Introduction

Over the past decade, living donors have become more and more important for kidney transplantation worldwide (1-3). Kidneys retrieved from living donors provide better function and longer half life after transplantation than kidneys from post-mortem donors (4-7). In the past, living kidney donors were strictly selected, and consequently represented the healthiest subjects of the population (8). Based on the growing need for organs, the selection criteria for potential donors, however, have become more liberal (9;10), with the inclusion of older, more overweight and even moderately hypertensive donors. Since aging, overweight and hypertension are well known risk factors for the development of kidney disease, these altered donor characteristics raise questions about the safety of living donation.

Due to the large reserve capacity of the kidney, minor interstitial, vascular and glomerular damage can already be present without clinical signs of deterioration such as proteinuria or decreased kidney function. In the single kidney situation following donation, pre-existent renal damage may, however, become of significant clinical importance. Several studies in potential donors showed that minor renal morphological damage, like glomerulosclerosis, interstitial fibrosis and tubular atrophy is present in subjects with good kidney function (11-16). The consequences in time of pre-existent damage for donor outcome are, however, unclear. It would, therefore, be of great interest to assess whether subclinical structural renal abnormalities have impact on renal outcome after donation. Associations have been described between pre-existent damage – like glomerulosclerosis, interstitial fibrosis and tubular atrophy – and decreased kidney function and lower graft survival in recipients of deceased donor kidneys (17-25).

In this study, we evaluate the effects of pre-existent morphological renal damage in renal biopsies taken during the donation procedure on donor characteristics prior to donation and the impact of these pre-existent renal morphological abnormalities on living donor short term renal function after donation.

Methods

Eighty-two living donors were included in this study. Biopsies from living donor kidneys retrieved between November 2005 and July 2008 at the University Medical Center Groningen were used. In this time period, 125 transplantations using living donors were performed. Nine of these donors were cross-over donors, and were screened at another university hospital in the Netherlands. Data of these screenings is unavailable. Screening or follow-up data from 13 donors were missing. Renal biopsies from 21 donors showed mere non-cortical tissue and were excluded from analyses. Kidney function was measured as the urinary clearance of 125I iothalamate four months prior and two months post donation. Kidney biopsies were taken at three time points: just before retrieval of the kidney (with the kidney in situ and before clamping arterial and venous circulation), at the end of cold ischemia and 45 min after reperfusion. For analysis, scores from all three time points were averaged; for the number of macrophages and neutrophillic granulocytes the third time point was excluded from analyses since reperfusion might enhance the influx of these cells. Procedures were conducted in accordance with the Helsinki declaration.

Kidney function measurements

Glomerular filtration rate (GFR) was measured by constant low-dose infusion of the radio-labeled tracer ^{125}I iothalamate as described by Visser and Apperloo et al. (26-28). Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. Filtration fraction (FF) was calculated as GFR/ERPF. For the measurements, subjects were seated in a quiet room in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%.

Morphological damage

Paraffin sections (3 µm) were stained with periodic acid-Schiff (PAS) to evaluate the degree of glomerulosclerosis, interstitial fibrosis (IF), arterial intima thickness and vascular hyalinosis. IF was defined as expansion of the interstitial space, with or without the presence of atrophied and dilated tubules and thickened tubular basement membranes. The renal papilla, glomeruli, and vessels were excluded from the calculated areas of fibrotic involvement. The degree of IF was scored on a scale of 5: 0%, 0-10%, 10-25%, 25-50%; 50-75% and 75-100% of biopsy surface area. A mean score was calculated from the three different time-points. Vascular hyalinosis was scored as none, mild, moderate, or nodular. A mean score was calculated from the three different time-points. At least three vessels had to be present to calculate a mean score. The renal biopsies were scored by a pathologist blinded for donor characteristics and clinical outcome.

Macrophage influx and α-Smooth muscle actin (α-SMA) expression

Deparaffinized sections were subjected to heat-induced antigen retrieval by overnight incubation in a 0.1 M Tris-HCl buffer (pH 9.0) at 80°C. Endogenous peroxidase (PO) was blocked with 0.3% H2O2 in PBS for 30 min, and sections were incubated with either CD68 antibody (clone PGM-1; diluted 1:250; DAKO, Glosstrup, Denmark) for macrophages or α-SMA antibody (clone 1A4, diluted 1:10,000, Sigma, Saint Louis, Missouri, USA) for 60 min at room temperature. Binding of the antibody was detected using sequential incubations (30 min each) with PO-labeled rabbit-anti-mouse (RAMPO, diluted 1:100; DAKO) and PO-labeled goat-anti-rabbit antibodies (GARPO, diluted 1:100, DAKO). PO activity was developed using 3,3'-diaminobenzidine tetrachloride (DAB) for 10 min. Sections stained for macrophages were counterstained with PAS. Macrophages were manually counted in the interstitium, α-SMA expression was determined by Positive Pixel Count (Aperio Imagescope version 10.2.2). Scores were adjusted for biopsy surface area. The renal papilla, glomeruli, and vessels were excluded

from counting and surface area calculation. For analysis of α-SMA expression, scores from the three time points (provided that all were cortical biopsies) were averaged. For analysis of the number of macrophages, the scores from the first and second time-point (both before reperfusion) were averaged.

Neutrophillic granulocytes
Deparaffinized sections were subjected to antigen retrieval using protease (diluted 1:1000; type XXIV, Sigma, Zwijndrecht, the Netherlands). Endogenous peroxidase (PO) was blocked with 0.3% H2O2 in PBS for 30 min, and sections were incubated with monoclonal antibody 12.8 (29) (clone 12.8; diluted 1:10) for 60 min at room temperature. Binding of the antibody was detected using sequential incubations (30 min each) with PO-labeled rabbit-anti-mouse and PO-labeled goat-anti-rabbit antibodies (both diluted 1:100). PO activity was developed using 3,3'-diaminobenzidine tetrachloride (DAB) for 10 min. Sections were counterstained with hematoxylin. Neutrophillic granulocytes were manually counted in the interstitium, scores were adjusted for biopsy surface area. For analysis, the scores from the first and second time-point (both before reperfusion) were averaged.

Arterial Intima thickness
To provide a standardized estimation of the severity of intima thickening independent of vessel size, intima surface was expressed as percentage of the media surface, in which a higher percentage represents a relatively thicker intima. For this purpose, all arteries present in the biopsies were analyzed. α-SMA stained sections were used for this purpose. For each vessel, media, and intima were outlined to calculate individual surfaces. For each donor, the separate vessel scores of all three biopsy time points were averaged to provide one overall score. At least three vessels had to be present to calculate an overall score.

Statistical analysis
Analyses were performed using SPSS software version 16.0 and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [IQR]. Donors with only medulla on their renal biopsy were excluded from analysis. To evaluate for associations of the damage parameters with donor characteristics and outcome, correlation analysis was used. Linear regression was used to evaluate the independency of associations found in correlation analysis from traditional predictors. The number of macrophages and neutrophillic granulocytes, the expression of α-SMA and the intima thickness showed a skewed distribution. For correlation and regression analysis, therefore, the natural logarithmic of these parameters was used.

Results
Donor characteristics are shown in table 1. After donation, GFR and ERPF fell to 63 and 65% of the pre-donation values. Sixteen donors (20%) used antihypertensive drugs, mainly angiotensin converting enzyme inhibitors (75%), prior to donation. Renal damage parameters are shown in table 2. Donors with interstitial fibrosis exceeding 10% of biopsy surface area

Table 1: Donor characteristics prior to and two months after donation.

	Value
N (%) female	82 (46)
Age at donationN	52 ± 11
BMI at donation (kg/m^2)	27 ± 4
Pre-donation	
Systolic blood pressure (mmHg)	129 ± 14
Diastolic blood pressure (mmHg)	76 ± 9
GFR (mL/min)	118 ± 23
ERPF (mL/min)	437 ± 85
FF (%)	27 ± 3
Urinary protein excretion (g/24h)	0.1 [0.0-0.2]
Two months post-donation	
Systolic blood pressure (mmHg)	125 ± 13
Diastolic blood pressure (mmHg)	77 ± 9
GFR (mL/min)	74 ± 15
ERPF (mL/min)	283 ± 53
FF (%)	26 ± 3
Urinary protein excretion (g/24h)	0.1 [0.0-0.2]
ΔGFR (mL/min)	-44 ± 14
ΔERPF (mL/min)	-154 ± 57

Values represent n(%), mean ± SD and median [IQR].

had higher interstitial α-SMA expression (4.3 [2.5-7.44] vs. 1.4 [0.6-2.4] per μm^2, p<0.01) and a higher number of interstitial macrophages (4.6 [3.2-7.6] vs. 1.2 [0.5-2.2] per μm^2, p<0.05).

Interstitial α-SMA expression

Interstitial α-SMA expression correlated positively to donor age at donation (R 0.32, p<0.01) and negatively to donor GFR and ERPF prior to donation (R -0.38 and -0.39, both p<0.01; figure 2). The associations with kidney function were both independent of donor age. There were no significant associations with donor gender or BMI. There was no difference in α-SMA expression between males and females, hypertensive and normotensive donors and donors with a BMI above or below 30 kg/m^2.

Interstitial α-SMA expression correlated negatively to early post-donation GFR and ERPF (R -0.36 and -0.41, both p<0.01), no other associations were found. Again, the associations

Table 2: Damage parameters at time of donation.

	Value
Interstitial α-SMA (intensity)	82 (46)
Interstitial macrophages (number)	52 ± 11
Interstitial neutrophillic granulocytes (number)	27 ± 4
Glomerosclerosis (% of total glomeruli)	
Interstitial fibrosis > 10% (n, %)	129 ± 14
Vascular hyalinosis (n,%)	76 ± 9
Arterial intima thickness (% of media thickness)	118 ± 23

Values represent n(%), mean ± SD or median [IQR]. The number of macrophages and neutrophillic granulocytes are expressed as number/10.000 μm^2 biopsy surface area. α-SMA expression is expressed as intensity/μm^2 biopsy surface area.

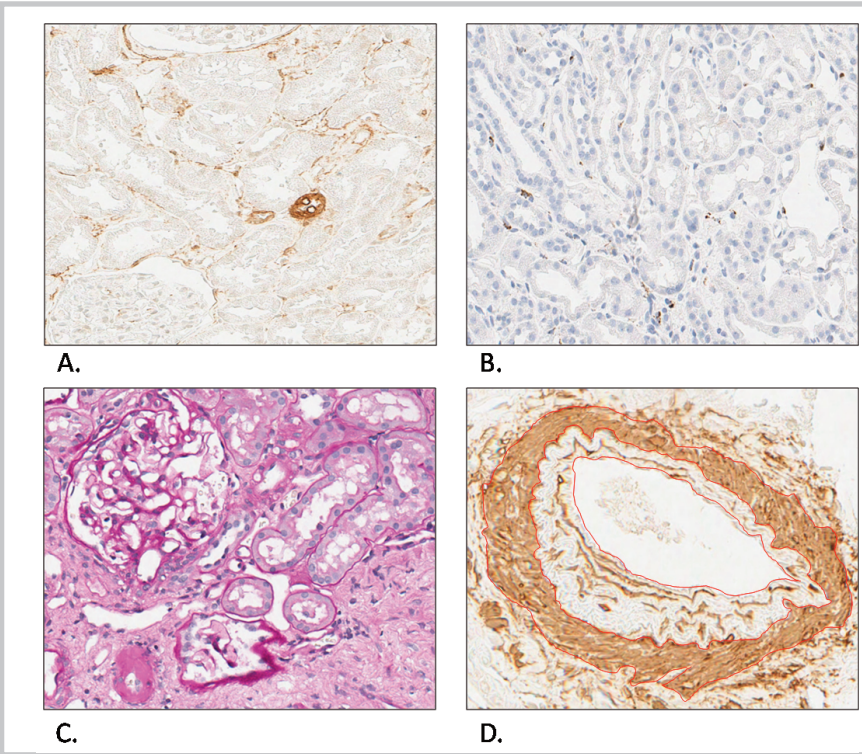

Figure 1: Representative photographs of kidney biopsies. A: α-SMA staining. B: CD68 staining. C. PAS staining showing vascular hyalinosis. D. α-SMA staining showing increased arterial intima thickness; red lines represent the borders of intima and media as determined for the calculation of the intima and media surface.

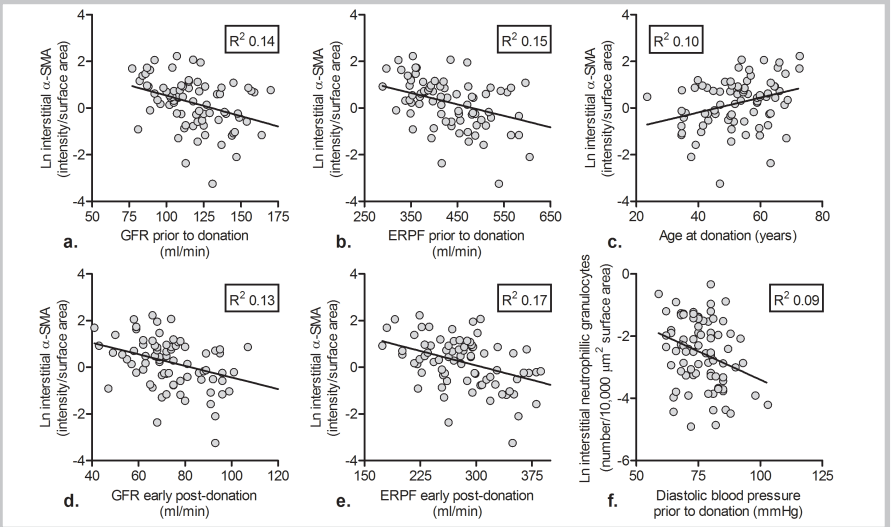

Figure 2: Scatter plots for the associations between damage markers and donor characteristics and outcome. Figures represent associations between Ln interstitial α-SMA expression and a) GFR prior to donation, b) ERPF prior to donation, c) age at donation, d) GFR early post-donation, e) ERPF early post-donation; and between f) Ln interstitial neutrophillic granulocytes and diastolic blood pressure prior to donation. R^2 represents adjusted R^2 by linear regression analysis. All associations were independent of donor age.

with post-donation GFR and ERPF were independent of donor age. There were no associations with the change in kidney function over donation.

Interstitial macrophages and neutrophilic granulocytes

The median number of interstitial macrophages and neutrophillic granulocytes is shown in table 2. The number of interstitial macrophages did not correlate to donor characteristics Nevertheless, donors with a BMI above 30 kg/m^2 had a higher density of interstitial macrophages than leaner donors (0.99 [0.75-1.59] vs. 0.75 [0.51-1.10] per 10,000 µm^2, $p<0.05$). No correlations were found between the number of macrophages and donor outcome.

The number of interstitial neutrophillic granulocytes was higher in women (0.10 [0.06-0.22] vs. 0.05 [0.02-0.20] per 10,000 µm^2, $p<0.05$), and associated negatively to donor systolic and diastolic blood pressure prior to donation (R -0.25 and -0.29, both $p<0.05$). There was no difference in the number of interstitial neutrophillic granulocytes between donors older and younger than 60 years, donors with a BMI above or below 30 kg/m^2, or between hypertensive and normotensive donors.

In multivariate analysis, the associations between the number of interstitial neutrophillic granulocytes and pre- and post-donation blood pressure were adjusted for donor gender and donor age. The association with pre-donation diastolic blood pressure was independent of donor gender and age (figure 2).

The number of interstitial neutrophillic granulocytes associated negatively post-donation diastolic blood pressure (R -0.24, $p<0.05$). This association, however, was dependent of donor

age and gender.

Glomerulosclerosis and interstitial fibrosis

The average number of sclerosed glomeruli is shown in table 2. The percentage of glomerulosclerosis showed no associations with donor characteristics or outcome. No differences were found between donors older and younger than 60 years, donors with a BMI above or below 30 kg/m^2, or between hypertensive and normotensive donors.

Eight donors had interstitial fibrosis in more than 10% of the surface area. These donors had higher diastolic blood pressure prior to donation (82 [70-86] vs. 75 [69-81] mmHg, $p<0.05$). No other differences in donor characteristics or outcome were found.

Vascular hyalinosis and intima thickness

Vascular hyalinosis was present in 13% of donors. Donors with vascular hyalinosis present in their biopsy had higher systolic blood pressure prior to donation (137 [129-150] vs. 128 [119-135] mmHg, $p<0.05$). Donors older than 60, with a BMI > 30kg/m^2 or with pre-existent hypertension had similar presence of hyalinosis as their younger, leaner or normotensive counterparts. There were no differences in outcome between donors with and without signs of hyalinosis.

Intima thickness showed no association to donor characteristics or donor outcome. Donors with a BMI >30 kg/m^2 had higher intima thickness scores than donors with a BMI < 30kg/m^2 ($p> 0.05$).

Prediction of donor outcome

Interstitial α-SMA expression associated negatively with post-donation kidney function, independently of donor age. When combined with pre-donation characteristics and kidney function, however, the significance of interstitial α-SMA expression was lost. Post-donation GFR was best predicted by pre-donation GFR and age at donation (std. β 0.68 and -0.27; adjusted R^2 0.68; $p<0.01$). Post-donation ERPF was best predicted by pre-donation ERPF and age at donation (std. β 0.66 and -0.27; adjusted R^2 0.63; $p<0.01$). Pre-donation BMI and blood pressure had no influence in the multivariate models.

No associations were found between the change in kidney function over donation and damage parameters.

Discussion

The most important finding of this study relates to the presence of mild pro-fibrotic, vascular and inflammatory damage in living kidney donors that does not influence adaption to the single kidney state. Renal interstitial damage, as evidenced by α-SMA expression and the number of neutrophillic granulocytes, shows associations with donor age, blood pressure prior to donation and kidney function prior to and early post-donation. However, no influence on change in kidney function over donation was seen, and, thus, these changes do not seem to affect adaptive capacity of the remaining kidney. The impact on post-donation outcome was lost when well-established determinants of post-donation outcome, namely pre-donation

GFR and age, were taken into account. Thus, within the time frame of this study, no negative impact of the structural abnormalities could be identified.

This is the first study that evaluates associations between changes in pre-implantation biopsies and living donor characteristics and early post-donation outcome with the use of gold standard kidney function measurements. In these thoroughly screened donors, whom presented with a good kidney function, mild vascular, pre-fibrotic and inflammatory changes were already present at the time of donation. Our findings are in line with other studies that evaluated potential living kidney donors (11-13;15;16). Rule et al. showed in a large series of living kidney donors the presence of age related nephrosclerosis, that was subclinical and was only detected by kidney biopsy (13). Others reported associations between morphological damage – such as interstitial fibrosis and glomerulosclerosis – with donor age, estimated kidney function and presence of hypertension or blood pressure as such (11;12;15).

Interstitial α-SMA expression is an early structural marker for interstitial stress. Peritubular fibroblasts under the influence of cytokines produced by either activated interstitial inflammatory cells or damaged tubular cells are transformed into extracellular matrix producing myofibroblasts. Interstitial α-SMA showed a positive association to donor age and a negative association with pre-donation kidney function. Although kidney function declines with ageing (30-33), the associations between α-SMA expression and pre-donation kidney function were independent of donor age. The association between interstitial α-SMA expression and age is in line with previous studies that focused on age related changes.

Although α-SMA expression associated with post-donation kidney function, no influence on the change in kidney function was found. Apparently, adaptive capacity is preserved in the presence of mild pre-fibrotic changes. Early adaptation is to a large extent determined by hemodynamic adaptation. Prior studies showed that pre-donation kidney function is the strongest predictor of post-donation kidney function. In the present study, the association between α-SMA expression and post-donation kidney function was lost when pre-donation kidney function was taken into account. In recipients, however, several studies did show associations between baseline damage and graft performance (15;17-25). Kidney transplantation enhances the effects of pre-existent damage, while changes that occur in the remnant kidney after donation overcome these effects. Furthermore, many of the abovementioned studies, were performed with deceased donor kidneys. Since living donors are selected for good health and kidney function, their kidneys are of higher quality than deceased donor kidneys (4-7). Thus, less damage is expected in living donor kidneys, which may explain the smaller effect of preexistent changes on living donor outcome.

Our study has some limitations, the most important being the relatively small sample-size and short duration of follow-up. Furthermore, part of the biopsies had to be rejected from analysis due to the absence of sufficient amount of cortical area. This was partly compensated by the fact that three biopsies were taken from each kidney covers the greater part of the quality problems. Although the single center character can be regarded as a limitation, it provided us with detailed information of all donors prior to and early after donation.

In conclusion, mild pre-existent renal damage characterized by pro-fibrotic and inflammatory vascular and interstitial changes are present in kidneys of well screened living

kidney donors at time of donation. Interstitial α-SMA expression associates with pre-donation donor age, and kidney function prior to and early post-donation. Nevertheless, no influence of pre-existent damage on adaptive capacity was found. Thus, early adaptive capacity following kidney donation is preserved in the presence of mild pre-fibrotic changes.

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Part three:
Effect of donor risk profile on recipient renal outcome

Chapter 8

Donor kidney adapts to body dimensions of recipient: no influence of donor gender on renal function after transplantation

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Abstract

Background: Female kidneys and kidneys from small donors have been suggested to perform worse after kidney transplantation. Here we evaluate impact of gender and body dimensions on post-transplantation GFR in living donor transplantation.

Methods: Evaluated were 293 donor-recipient pairs who were transplanted at our centre. All pairs had detailed renal function measurement (^{125}I-iothalamate; ^{131}I-hippuran) 4 months pre-donation in the donor, and 2.5 months post-transplantation in donor and recipient. For 88 pairs five year recipient follow-up was available. Delta GFR was calculated as [recipient GFR – donor single kidney GFR].

Results: Recipients of both male and female kidneys had similar renal function at early and long term after transplantation. Male recipients had higher ERPF, ΔGFR and ΔERPF at both time points. Kidneys of donors smaller than their recipient had higher ΔGFR and ΔERPF than kidneys of larger donors at both time points ($p<0.05$). In multivariate analysis, ΔGFR was predicted by donor/recipient BSA-ratio together with transplantation related factors (R^2 0.19) irrespective of donor and recipient gender.

Conclusion: In living donor transplantation, female kidneys perform as well as male donor kidneys. Kidneys adapt to the recipients body size and demands independent of gender, without detrimental effects in renal function and outcome up to mid-long term.

Introduction

Kidney transplantation is the preferred treatment for end-stage renal disease. It has been suggested that female kidneys perform worse after renal transplantation compared to male kidneys, especially when transplanted in a male recipient (1;2). This could be due to the fact that women generally are smaller than men, with consequently smaller kidneys, less nephron mass (3) and lower GFR, i.e. quantitative differences in the filtering capacity of the transplanted kidney. Several studies showing impact of donor body dimensions on transplant outcome support this assumption (4-11). Alternatively, intrinsic differences between male and female kidneys might play a role, as in native kidneys many differences in renal physiology and pathophysiology have been described between men and women (12-14), that may be relevant to outcome after transplantation (15).

The living donor program, with detailed data on donor characteristics including renal function before donation, provides an excellent setting to determine the effects of body dimensions on renal function after transplantation from those of gender. Therefore, in the current study we evaluated the effect of donor gender, donor body dimensions and the associated differences in renal function on short- and long-term renal function of the recipient, in relation to recipient gender and body dimensions.

Methods

In this study a total of 293 consecutive couples of living donors and their recipients, who were transplanted in the University Medical Center Groningen, were included. Glomerular filtration rate (GFR) was measured as described below four months prior and two months after donation for the living donors and a median of 2.5 [0.3] months for the recipients. For 88 couples 5 year recipient follow-up was available as well. Couples not available for 5 year follow-up had higher donor and recipient BMI, and higher pre-donation donor GFR than couples with 5 year follow-up. There were no significant differences in demographics such as donor and recipient age, recipient GFR and GFR/BSA, donor GFR/BSA and recipient urinary protein excretion early after transplantation (data not shown). Procedures were conducted in accordance with the Helsinki declaration.

GFR measurement

GFR was measured by constant low-dose infusion of the radio-labelled tracer ^{125}I iothalamate as described by Visser and Apperloo et al. (16;17). Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. Filtration fraction (FF) was calculated as GFR/ERPF. For the measurements, subjects were seated in a quiet room in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer

and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%. Next to basal function, renal reserve capacity was measured pre- and two months post-donation as part of the screening and early follow-up. To obtain reserve capacity, the above-mentioned baseline procedure was extended for two hours. During this period, dopamine was infused at a rate of 1.5 µg/kg per minute.

Treatment regimen after transplantation

Standard immunosuppression consisted of the following: cyclosporine standard formulation (Sandimmune; Novartis Pharma b.v., Arnhem, The Netherlands; 10 mg/kg; trough levels of 175–200 mg/L for the first three months, 150 mg/L between three and twelve months posttransplant and 100 mg/L thereafter) combined with prednisolone (starting with 20 mg/day, rapidly tapered to 10 mg/day) from January 1988 to February 1993. Cyclosporine microemulsion (Neoral; Novartis Pharma b.v., Arnhem, The Netherlands; 10 mg/kg; trough levels idem) and prednisolone from March 1993 to May 1997. Mycophenolate mofetil (Cellcept; Roche b.v., Woerden, The Netherlands; 2 g/day) was added from May 1997 to date.

Calculations

Body surface area (BSA) was calculated as according to DuBois (18). GFR was normalized by dividing the raw sample by BSA and multiplying it with 1.73, giving GFR/BSA. Mean arterial pressure (MAP) was calculated as: MAP = ((2 * diastolic pressure) + systolic pressure)/3. BSA-ratio was calculated as: donor BSA /recipient BSA. Delta GFR from donor to recipient was calculated as: recipient value - (donor pre-donation value /2). The donor value/2 represents a 'single kidney' value (GFR_{SK}). Delta GFR from single kidney status was calculated for short term recipient outcome (ΔGFR_{ST}), and long term post-transplantation outcome (ΔGFR_{LT}).

Statistical analysis

Analyses were performed using SPSS software version 16.0 and GraphPad Prism version 5. Data are given as mean ± standard deviation or median [IQR]. Independent samples t Test, One-Way ANOVA and Mann-Whitney U test were used to analyse for differences between groups. To determine impact of the balance between donor and recipient BSA, data was analysed by a break-up by donor/recipient BSA-ratio <1 or ≥1. To evaluate which transplantation related factors were associated with ΔGFR_{ST} and $\Delta ERPF_{ST}$, we performed univariate regression analysis. Factors with a P-value ≤0.1 in univariate analysis were entered into a backward linear regression , combined with donor and recipient gender, BSA-ratio and 24 hour creatinine excretion, and removed in successive steps at a threshold of P-value ≤0.05.

Results

Renal function by donor gender

Donor characteristics by a break-up in gender are shown in table 1. Pre- and post-donation, male donors had higher BSA, MAP, GFR and ERPF. After correction for BSA, renal function was

similar in men and women. The adaptive response in renal function to nephrectomy was similar for males and females. For couples with five year follow-up, donor characteristics were similar, except there was no gender difference in MAP (data not shown). For a subset of donors (270 pre-donation and 256 early pos-donation), data on renal reserve capacity was available as well. As expected, the change in GFR in response to dopamine was lower post-donation than pre-donation (12 ± 24 vs. 4 ± 18; p<0.01). There were no differences in reserve capacity between male and female donors, and donors with BSA-ratio <1 or ≥1. Reserve capacity did not correlate to donor BSA.

Transplantation related factors, evolution after transplantation, drug regimen and use of antihypertensive drugs are listed in table 2, as well as univariate associations between these factors and ΔGFR_{ST} and $\Delta ERPF_{ST}$. There were no differences for kidneys of male and female origin (data not shown). Male recipients had a higher tacrolimus through level, higher total number of antihypertensive drugs and more often used a calcium channel blocker (all p<0.05).

Table 1: donor characteristics prior and early after donation.

	Total	Male	Female	*P* value
Pre-donation				
N	293	131	162	-
Age (years)	50 ± 11	50 ± 12	49 ± 9	NS
BMI (kg/m^2)	26 ± 4	26 ± 4	26 ± 4	NS
BSA (m^2)	1.92 ± 0.20	2.06 ± 0.16	1.81 ± 0.15	<0.001
MAP (mmHg)	93 ± 9	95 ± 9	91 ± 9	<0.001
GFR (mL/min)	115 ± 20	123 ± 19	108 ± 19	<0.001
ERPF (mL/min)	430 ± 84	455 ± 86	409 ± 76	<0.001
GFR/BSA (mL/min/1.73m^2)	103 ± 15	103 ± 15	104 ± 16	NS
ERPF/BSA (mL/min/1.73m^2)	387 ± 71	382 ± 70	392 ± 72	NS
Post-donation				
MAP (mmHg)	94 ± 10	96 ± 9	92 ± 9	<0.001
GFR (mL/min)	73 ± 13	77 ± 13	69 ± 12	<0.001
ERPF (mL/min)	279 ± 54	95 ± 55	266 ± 49	<0.001
GFR/BSA (mL/min/1.73m^2)	66 ± 10	65 ± 10	66 ± 10	NS
ERPF/BSA (mL/min/1.73m^2)	252 ± 46	249 ± 46	254 ± 46	NS
ΔGFR (mL/min)	15 ± 8	16 ± 9	15 ± 8	NS
ΔERPF (mL/min)	64 ± 31	68 ± 33	61 ± 29	NS

Values represent mean ± SD. p-Values in table represent male compared to female values. ΔGFR: post-donation GFR – pre-donation single kidney GFR; ΔERPF: post-donation ERPF– pre-donation single kidney ERPF.

Table 2: transplantation related characteristics, evolution post-transplantation and drug use for the whole group (middle column, n=293) and the group with five year follow-up (right column, n=88).

		ΔGFR_{ST}		$\Delta ERPF_{ST}$		5 year follow-up value
	Value	Std. β	*P* value	Std. β	*P* value	
Primary renal disease (n, %)						
Primary glomerular disease	26 (9)	Ref	Ref	Ref	Ref	9 (10)
Glomerulonephritis	78 (27)	0.07	0.24	0.18	<0.01	25 (28
Tubular interstitial disease	9 (3)	-0.06	0.39	-0.11	0.10	0 (0)
Polycystic kidney disease	53 (18)	0.04	0.57	-0.08	0.22	14 (16)
Dysplasia and hypoplasia	45 (16)	-0.04	0.55	0.05	0.41	16 (18)
Renovascular disease	33 (11)	-0.05	0.39	0.09	0.15	11 (13)
Diabetes Mellitus	10 (3)	0.04	0.53	0.05	0.40	3 (4)
Other or unknown cause	39 (13)	-0.03	0.60	-0.05	0.40	10 (11)
Related donor (n, %)	174 (59)	-0.01	0.84	-0.10	0.13	60 (68)
Pre-emptive procedure (n, %)	97 (33)	0.05	0.44	0.11	0.10	19 (22)
Previous transplant (n, %)	14 (5)	-0.07	0.30	-0.10	0.14	7 (8)
Warm ischemia times (min)	44 ± 18	-0.05	0.42	0.05	0.44	36 ± 17
Cold ischemia times (min)	156 ± 103	-0.05	0.45	0.01	0.82	160 ± 93
HLA-AB mismatches (n)	1.9 ± 1.1	0.07	0.28	0.05	0.48	1.8 ± 1.2
HLA-DR mismatches (n)	1.0 ± 0.7	-0.05	0.44	-0.03	0.70	0.8 ± 0.7
Panel reactive antibodies 0-5 % (n, %)	255 (87)	-	-	-	-	76 (86)
Panel reactive antibodies >5 % (n, %)	38 (13)	-	-	-	-	12 (14)
Delayed graft function (days)	0 [0-0]	-	-	-	-	0 [0-0]
Acute rejection (n, %)	77 (26)	-0.20	<0.01	-0.18	0.01	61 (69)
Drug use						
Cyclosporine (n, %)	233 (80)	0.07	0.27	0.07	0.21	34 (39)
Trough-level (µg/L)	203 ± 73	-0.08	0.25	-0.15	0.04	97 ± 44
Tacrolimus (n, %)	60 (20)	-0.08	0.19	-0.07	0.24	19 (22)
Trough-level (µg/L)	13 ± 5	0.12	0.40	0.02	0.91	8 ± 4
Prednisolone dose (mg/day)	10 ± 1	0.12	0.07	0.06	0.41	9.1 ± 1. 4
Azathioprine (n, %)	10 (3)	0.02	0.77	0.11	0.07	12 (14)
Mycophenolate mofetil (n, %)	268 (91)	0.14	0.02	0.15	0.01	71 (81)
Number of antihypertensives	1.3 ± 0.9	-0.06	0.38	0.06	0.41	1.3 ± 0.9
ACE-inhibitor or AII-antagonist (n, %)	65 (22)	0.08	0.24	0.12	0.08	39 (44)
β-blocker	164 (56)	-0.04	0.52	-0.03	0.65	59 (67)
Calcium channel blocker	82 (28)	0.21	<0.01	0.27	<0.01	21 (24)
Statin	113 (39)	-0.09	0.20	-0.05	0.44	41 (47)
Total cholesterol (mmol/L) %)	5.4 ± 1.1	-0.17	0.01	-0.19	0.02	5.2 ± 1.2

Values represent mean ± SD and median [IQR]. Standardized β (Std. β) and *P*-value were obtained by univariate regression analysis with either ΔGFR_{ST} or $\Delta ERPF_{ST}$ as dependent variable.

Table 3: short term and 5 year recipient outcome by a break-up by donor gender.

	Total	Male donor	Female donor	*P* value
Short term recipient outcome				
N	293	131	162	-
Male recipients (%)	60	45	73	<0.01
Age (years)	44 ± 15	43 ± 16	44 ± 14	NS
BSA (m^2)	1.93 ± 0.21	1.91 ± 0.21	1.95 ± 0.21	NS
Creatinine excretion (μmol/24h)	12.4 ± 3.6	11.9 ± 3.5	12.9 ± 3.6	0.029
MAP (mmHg)	104 ± 12	103 ± 12	105 ± 11	NS
GFR (mL/min)	59 ± 17	61 ± 18	58 ± 16	NS
ERPF (mL/min)	239 ± 64	247 ± 66	233 ± 62	NS
GFR/BSA (mL/min/1.73m^2)	53 ± 16	56 ± 17	52 ± 15	0.033
ERPF/BSA (mL/min/1.73m^2)	216 ± 60	224 ± 61	209 ± 58	0.027
FF (%)	25 ± 5	25 ± 4	25 ± 5	NS
UPE (g/24h)	0.3 [0.2-0.4]	0.2 [0..02-0.4]	0.3 [0.2-0.5]	<0.01
ΔGFR (mL/min)	2 ± 16	0 ± 15	3 ± 16	0.033
ΔERPF (mL/min)	23 ± 58	19 ± 56	27 ± 60	NS
Five year recipient outcome				
N	88	31	57	NS
Male recipients (%)	65	58	68	NS
Age (years)	46 ± 14	43 ± 13	46 ± 14	NS
BSA (m^2)	1.95 ± 0.19	1.98 ± 0.20	1.94 ± 0.18	NS
Creatinine excretion (μmol/24h)	12.7 ± 3.7	13.1 ± 4.1	12.5 ± 3.6	NS
MAP (mmHg)	100 ± 11	100 ± 12	101 ± 11	NS
GFR (mL/min)	57 ± 18	55 ± 23	57 ± 16	NS
ERPF (mL/min)	223 ± 59	213 ± 66	229 ± 54	NS
GFR/BSA (mL/min/1.73m^2)	50 ± 16	48 ± 19	51 ± 14	NS
ERPF/BSA (mL/min/1.73m^2)	200 ± 51	191 ± 55	204 ± 49	NS
FF (%)	25 ± 5	26 ± 6	25 ± 4	NS
UPE (g/24h)	0.2 [0.0-0.4]	0.2 [0.0-0.4]	0.2 [0.0-0.4]	NS
ΔGFR (mL/min)	1 ± 17	-3 ± 20	3 ± 15	NS
ΔERPF (mL/min)	9 ± 58	-9 ± 53	19 ± 58	0.032

Values represent mean ± SD and median [IQR]. P-Values in table represent male compared to female values.
UPE: urinary protein excretion; ΔGFR: recipient GFR – donor single kidney GFR.

Female recipients more often used a statin, and had higher percentages of panel reactive antibodies (PRA; p<0.01).

Recipient renal function by a break-up in donor gender is shown in table 3. Among the recipients of a female kidney the majority was male (p<0.01 compared to recipients of male donors), as also reflected by a higher BSA and 24 hour creatinine excretion among these recipients. Early after transplantation, mean GFR and ERPF were similar for recipients of male

Table 4: short term and 5 year recipient outcome by a break-up by recipient gender.

	Total	Male recipient	Female recipient	*P* value
Short term recipient outcome				
N	293	177	166	-
Male recipients (%)	45	33	62	<0.01
Age (years)	44 ± 15	43 ± 14	44 ± 15	NS
BSA (m^2)	1.93 ± 0.21	2.00 ± 0.18	1.83 ± 0.22	<0.01
Creatinine excretion (µmol/24h)	12.4 ± 3.6	13.7 ± 3.4	10.5 ± 2.9	<0.01
MAP (mmHg)	104 ± 12	105 ± 11	103 ± 12	NS
GFR (mL/min)	59 ±17	60 ± 17	58 ± 16	NS
ERPF (mL/min)	239 ± 64	246 ± 66	229 ± 59	0.03
GFR/BSA (mL/min/1.73m^2)	53 ± 13	52 ± 15	56 ± 16	NS
ERPF/BSA (mL/min/1.73m^2)	216 ± 60	214 ± 62	218 ± 56	NS
FF (%)	25 ± 5	25 ± 5	26 ± 4	NS
UPE (g/24h)	0.3 [0.2-0.4]	0.3 [0.2-0.5]	0.2 [0.0-0.4]	NS
ΔGFR (mL/min)	2 ± 16	4 ± 16	-2 ± 15	<0.01
ΔERPF (mL/min)	23 ± 58	33 ± 59	9 ± 55	<0.01
Five year recipient outcome				
N	88	57	31	-
Male recipients (%)	35	32	42	NS
Age (years)	46 ± 14	45 ± 14	49 ± 12	NS
BSA (m^2)	1.95 ± 0.18	2.02 ± 0.14	1.84 ± 0.20	<0.01
Creatinine excretion (µmol/24h)	12.7 ± 3.7	14.1 ± 3.6	10.1 ± 2.4	<0.01
MAP (mmHg)	100 ± 11	101 ± 10	100 ± 13	NS
GFR (mL/min)	57 ± 18	59 ± 20	53 ± 15	NS
ERPF (mL/min)	223 ± 59	233 ± 60	205 ± 53	NS
GFR/BSA (mL/min/1.73m^2)	50 ± 16	51 ± 16	49 ± 16	NS
ERPF/BSA (mL/min/1.73m^2)	200 ± 51	203 ± 48	195 ± 58	NS
FF (%)	25 ± 5	25 ± 5	26 ± 6	NS
UPE (g/24h)	0.2 [0.0-0.4]	0.2 [0.0-0.5]	0.0 [0.0-0.2]	<0.01
ΔGFR (mL/min)	1 ± 17	4 ± 17	-5 ± 15	0.01
ΔERPF (mL/min)	9 ± 58	23 ± 59	-16 ± 47	<0.01

Values represent mean ± SD and median [IQR]. P-Values in table represent male compared to female values.
UPE: urinary protein excretion; ΔGFR: recipient GFR – donor single kidney GFR.

and female donor kidneys. The rise in single kidney GFR (ΔGFR_{ST}) was higher in kidneys of female origin, but the resulting GFR/BSA and ERPF/BSA were higher in kidneys of male origin. Recipients of a female kidney had slightly higher urinary protein excretion at this point in time.

Five year post transplantation no difference in renal function between recipients of male and female kidneys was observed, neither for the nominal value, nor after correction for

TabTable 5: recipient renal function short and long term after transplantation by a break-up in BSA-ratio. BSA-ratio < 1 reflects a couple with a donor smaller than its recipient and vice versa.

	Short term outcome			Five year outcome		
BSA ratio	<1	≥1	*P* value	<1	≥1	*P* value
N	146	147	-			-
GFR (mL/min)	59 ± 17	60 ± 18	NS	59 ± 19	54 ± 17	NS
GFR/BSA (mL/min/1.73m^2)	49 ± 14	57 ± 16	<0.01	51 ± 16	50 ± 16	NS
ERPF (mL/min)	238 ± 63	242 ± 67	NS	228 ± 61	218 ± 56	NS
ERPF/BSA (mL/min/1.73m^2)	201 ± 54	231± 62	<0.01	197 ± 50	203 ± 53	NS
ΔGFR (mL/min)	3.7± 16	-0.2 ± 15	0.03	4.4 ± 17	-3.0 ± 16	0.04
ΔGFR (mL/min)	32 ± 59	15 ± 57	0.02	20 ± 62	-3.0 ± 51	0.06
FF (%)	25 ± 4	24 ± 5	NS	25 ± 4	25 ± 6	NS
MAP (mmHg)	105 ± 15	102 ± 12	NS	101 ± 11	100 ± 12	NS
UPE (g/24h)	0.3 [0.2]	0.2 [0.3]	<0.01	0.2 [0.2]	0.2 [0.4]	NS

Values represent mean ± SD or median [IQR]. P-Values in table represent BSA-ratio<1 compared to BSA-ratio ≥ 1 values. BSA-ratio: donor BSA / recipient BS; ΔGFR: recipient GFR – donor single kidney GFR; UPE: urinary protein excretion.

recipient BSA. However, the rise from pre-donation values for ERPF ($\Delta ERPF_{LT}$) was larger in kidneys from female origin. A corresponding difference for GFR did not reach statistical significance at five year outcome.

Renal function by donor and recipient gender

To evaluate the effect of recipient gender on recipient renal function, table 4 shows recipient renal function by a break-up in recipient gender. Male recipients received a kidney of female origin more often than female recipients (p<0.01). Male recipients were generally larger than female recipients, reflected by a higher BSA and 24 hour creatinine excretion. Although crude ERPF was higher in male recipients, renal function corrected for BSA was similar between men and women. However, the rise from pre-donation values for GFR and ERPF (ΔGFR_{ST} and $\Delta ERPF_{ST}$) was larger in male recipients.

Five year post-donation a similar pattern was seen. Furthermore, male recipients showed higher urinary protein excretion than female recipients.

Impact of donor and recipient body surface area and their ratio on renal function

Female donors donate more often to male recipients and vice versa, which results in a relatively large body size disparity between donor and recipient. To analyse for the effect of body size disparity on recipient outcome we calculated donor/recipient BSA ratio. Mean donor to recipient BSA ratio for female donors was 0.97 ± 0.12 compared to 1.06 ± 0.13 for male donors (p<0.01). Table 5 shows recipient renal function early and late after transplantation according to BSA-ratio. Both early and long term after transplantation, the net performance of kidneys from donors who are smaller than their recipient (BSA-ratio <1) was similar to kidneys from donors larger than their recipients. Early after donation, GFR/BSA and ERPF/BSA were significantly lower in recipients of a kidney from a small donor. This difference was not

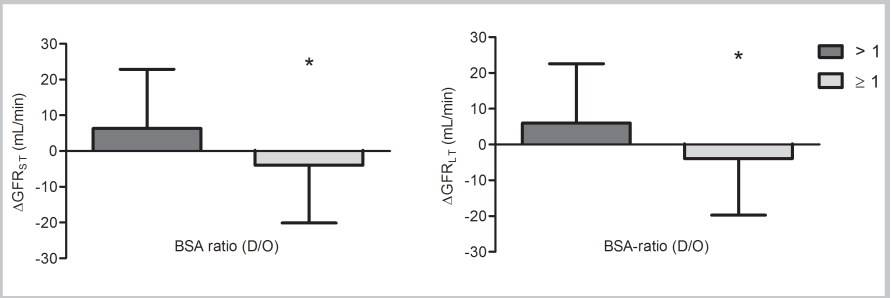

Figure 1: Change in single kidney GFR early after donation (short term ΔGFR_{ST}; left panel) and 5 year after donation (ΔGFR_{LT} right panel) by a break-up by the ratio of donor to recipient BSA. (BSA-ratio) *: p<0.05 for difference between the groups with BSA ratio < 1 (donor < recipient) vs. BSA-ratio >1 (donor > recipient).

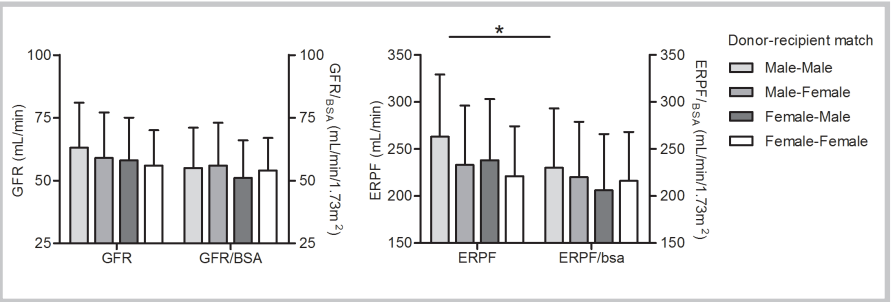

Figure 2: Short term recipient outcome by a break-up in donor and recipient gender. Categories represent donor to recipient match. *p<0.01 (ANOVA).

found any more at five years. The change in renal function from pre-donation values was significantly larger in kidneys from donors smaller than their recipients for both GFR and ERPF, for the short and long term (table 5, figure 1). Also, this was associated with slightly higher urinary protein loss early after transplantation (p<0.01), that was no longer apparent on five year follow-up. There were no differences in the occurrence of rejection, graft failure or death of the recipient between the groups (data not shown). These data indicate that kidneys from donor with smaller body dimensions increase there function after transplantation more than kidneys of larger donors.

Combined impact of donor and recipient gender and body size dimensions on recipient outcome

Since the abovementioned results might be explained by either gender differences or differences in body size dimensions between the genders or their combination, we also analysed for the combined effects of these factors.

Short term recipient renal function by a break-up in donor and recipient gender is shown in figure 2. Although ERPF showed a decrease over the categories (p<0.01, ANOVA), ERPF/BSA was similar, as were GFR and GFR/BSA.

To analyse for the combined effect of donor and recipient gender and body size

dimensions we used backward linear regression. Donor and recipient gender, BSA-ratio and 24 hour creatinine excretion were entered in the model. To adjust for transplantation related factors, all factors with a P-value ≤0.1 in univariate analysis (table 2) were entered in the backward regression model as well. For ΔGFR_{ST} the model consisted of BSA-ratio (standardized [std.] β -0.20), occurrence of rejection (std. β -0.16), prednisolone dose (std. β 0.17), use of mycophenolate mofetil (std. β 0.19), use of a calcium channel blocker (std. β 0.18) and total cholesterol level (std. β -0.16); adjusted R^2 0.19, $p<0.01$. For $\Delta ERPF_{ST}$ the model consisted of BSA-ratio (std. β -0.24), cyclosporine through level (std. β -0.26), pre-emptive character of the procedure (std. β 0.15), use of calcium channel blocker (std. β 0.32) and total cholesterol level (std. β -0.17); adjusted R^2 0.27, $p<0.01$. In both models, donor and recipient gender were lost as predictors.

For long term recipient renal function, the group sizes for the different gender combinations were too small to perform backward regression analysis. In linear regression, the strongest prediction of ΔGFR was provided by BSA-ratio (adjusted R^2 0.08, $p<0.01$). For ΔERPF, the strongest prediction was obtained by 24 hour creatinine excretion (adjusted R2 0.09, $p<0.01$).

Discussion

In this study we demonstrate that female kidneys perform as well as male kidneys after living kidney donation. Gender effects on recipient outcome can be explained by differences in body size between the sexes. The balance between donor and recipient body size appears to play a more important role than gender differences. Grafts of donors smaller than their recipients have a lower nominal GFR before donation, but increase their function after transplantation more in response to the recipient body dimensions than grafts from a larger donor. Thus, the kidney adapts to recipient body size, independent of donor and recipient gender.

This study is the first to evaluate effects of gender and body size disparity in living kidney donation with measurement of GFR in donor and recipient, in the short- and long-term follow-up. Previous studies on the effects of body and renal size disparity in renal transplantation focused mainly on hard end-points such as delayed graft function, donor and graft survival, and recipient estimated renal function (eGFR). Here we chose to evaluate recipient renal function and especially post-transplantation gain in renal function calculated from the renal function before donation. Our living kidney donor program provides us with detailed data on donor renal function. Unfortunately, we have no data on graft weight. Therefore, we used donor BSA as a substitute, since BSA has been shown to have a good correlation with kidney weight (19). Also, in this study we showed a strong relation between donor BSA and renal function.

In our population, body size disparity was shown to have substantial effects on gain in graft function after transplantation, independent of donor and recipient gender. Kidneys derived from donors smaller than their recipient gain more renal function than kidneys from larger donors which results in similar renal function in the long term. Thus, the kidney is able to adapt to its new body size, living up to the new metabolic demands. This does not seem to occur at the expense of hyperfiltration, since no difference in filtration fraction was

observed (table 4). Rather, adaptation occurs proportionally for perfusion and filtration, as shown from the stable filtration fraction. Probably, the superior quality and low damage impact in the living transplant kidney enables the graft to serve demand by an increase in perfusion and hence filtration, rather than filtration pressure. Furthermore, in the remnant donor kidney the capability to respond to dopamine post-donation is preserved, suggesting that the adaptative response in the donor has not pushed the kidney to the limits of its reserve capacity. However, in the short term a slightly higher urinary protein excretion was seen, which may reflect short term stress from a graft which is forced to meet the recipient demands. In the interpretation of renal hemodynamics, however, it should be noted that transplantation related factors, like the use of cyclosporine, could impact on renal hemodynamics and thus mask, or annihilate a possible hyper filtration pattern. For neither short- nor long-term were differences seen in absolute renal function between recipient with a BSA-ratio <1 and ≥1.

Previous studies have demonstrated a lower (graft) survival and lower renal function in recipients receiving a female kidney (1;2;7;20). Others showed a definite influence of recipient gender (21;22). Gender differences were explained by higher metabolic demands in males (10;22), and graft/recipient weight ratio (4;6;7;10;11). In our study, we attribute gender-related differences to differences in body size and thus BSA. Although previous studies showed that BSA indexing cannot resolve gender differences (17;23), our indexing of GFR for BSA did result in similar renal function for both sexes.

Studies on the effects of body size disparity have found more delayed graft function, lower renal function, higher mortality and graft loss due to differences in body size in deceased (6;8;9) and living donor transplantation (5;11). On the other hand, some studies did not show any effect on medium to long-term outcome (22;24;25). Differences between our and previous findings may be explained by the use of actual GFR measurement instead of calculation of eGFR or creatinine clearance. The relatively short duration of follow-up may play a role as well. Giral et al. showed detrimental effects of a low kidney weight to recipient weight ratio on long-term renal function, when effects were first seen after seven years of follow-up (6). Another explanation could be that we restricted analyses to recipients of a living donor kidney. Due to better organ viability compared to deceased donor kidneys with less ischemia-reperfusion injury, recipient outcome of living kidney donor organs is superior to outcome of deceased donor organs. This low damage impact and superior quality could mask gender and body size-related effects.

In this study we saw no detrimental effects of mismatch in gender or body size at the five year follow-up. However, we do find slightly higher urinary protein excretion in kidneys of small donors early after transplantation. This may be an early sign of a maladaptive response, but this difference is lost at five year. Possibly the living kidney can overcome the stress of the initial response to injury related to retrieval, short preservation and reperfusion at time of transplantation. Further damage may occur many years after transplantation. Thus, more reprised analyses are needed to elucidate effects of body size and gender disparity on the very long term.

Limitations of this study are the homogenous population, the single center character and the limited sample size for the long term follow-up. Overall, donors and recipient were only

slightly overweight and only 11% of donors was older than 60 years at time of donation. This hampers the generalizability of our data to populations where weight excess is more common among donors, or to populations with a higher donor age. Furthermore, generalizability to populations with endemic metabolic disorders is not feasible.

In summary, this study shows that with respect to recipient renal function female kidneys perform as well as male kidneys in living kidney donation. Kidneys of living donors adapt to the recipient's body size and demands without detrimental effects in renal function and outcome up to mid-long term.

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Chapter 9

Influence of living donor risk profile on recipient renal outcome

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Abstract

Background: Due to more liberal donor selection, current living kidney donors are older, more overweight and even hypertensive, which might impact recipient outcome. Here we evaluate the influence of the pre-donation donor risk profile on recipient renal outcome.

Methods: Evaluated were 113 donor-recipient pairs. All had GFR measurements (^{125}I-iothalamate) and ERPF measurements (^{131}I-hippurate) one year and five year post-transplantation.

Results: Overall recipient GFR at one and five year was good (59±15 and 58±19 mL/min). Donor GFR and ERPF associated positively to recipient GFR and ERPF at one and five years post-transplantation (R 0.30 and 0.39 for GFR, R 0.36 and 0.32 for ERPF). Donor age associated negatively to recipient GFR and ERPF at five year post-transplantation (R -0.24 and -0.20). Donor BMI, blood pressure and smoking behavior were not associated to recipient outcome. There were no differences in one and five year GFR and ERPF between recipients of older, overweight, hypertensive or smoking donors compared to recipients of younger, normal weigh, normotensive or non smoking donors.

Conclusion: In a well-screened population the donor risk profile exerts only a modest effect on recipient outcome in the medium term. This provides opportunities to expand the living donor pool without adverse effect on recipient outcome.

Introduction

Living kidney donors have become increasingly important in kidney transplantation. To enlarge the donor pool, selection of potential donors became more liberal. Previously, potential donors had to live up to strict selection criteria, and represented the healthiest part of the population (1). Kidney donors nowadays, however, are older, more overweight, and even slightly hypertensive (2). These characteristics are all established risk factors for the development of kidney function impairment. Higher donor age is a risk factor for worse kidney function and worse transplant outcome for transplants from deceased donors. Moreover, higher donor age negatively influences recipient estimated kidney function in living kidney donation (3-6). Hypertension (7-10), overweight (11-13) and smoking (14-19) are also associated with an increased risk for progressive kidney function loss in native as well as transplanted kidneys. However, there is no data on the impact of hypertension, overweight and smoking in the living donor on kidney function and transplant outcome in the recipients.

Therefore, in the current study, we evaluate the influence of the living donor risk profile, in particular with respect to age, blood pressure, smoking behavior and body mass index (BMI), on recipient kidney function one and five year post-transplantation, in a single center cohort.

Methods

We evaluated 113 pairs of living donors and their recipients, who were transplanted in the University Medical Center Groningen between 1988 and 2006. Kidney function was measured as described below, four months prior to donation for the donors, and one and five years after transplantation for the recipients. Procedures were conducted in accordance with the Helsinki declaration.

Routine donor screening

In donors, the basal kidney function measurement is extended by two hours in which the kidney function is stimulated by dopamine, to measure the so called reserve capacity. Donors were eligible to donate with a glomerular filtration rate (GFR) > 80 mL/min. For older subjects or small females, an exception could be made based on a good response to dopamine infusion, with a stimulated GFR > 80 mL/min. Since 2002, donors who use antihypertensive drugs are allowed to donate. There is no maximum for age. A maximum of two antihypertensive drugs is tolerated, provided ambulatory blood pressure does not exceed 150/85 mmHg. Blood pressure was measured for 30 minutes during kidney function measurement, with a semi-automated device (Dinamap; Criticon Inc, Tampa, FL). Donors with a body mass index (BMI) exceeding 30 kg/m^2 are encouraged to lose weight. Potential donors with a disturbed glucose tolerance test are rejected, as well as donors with severe atherosclerotic lesions. Smoking behavior has never been a selection criterion at our center.

Kidney function measurement

Glomerular filtration rate (GFR) was measured by constant low-dose infusion of the radio-labelled tracer ^{125}I iothalamate, as originally described by Donker, and more recenly by Visser et al. (20-22). Simultaneously, effective renal plasma flow (ERPF) was measured as the clearance of ^{131}I-hippurate. For the measurements, subjects were seated in a quiet room in, in a semi-supine position. After drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution, (0.04 MBq of ^{125}I iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra 0.6 MBq of ^{125}I iothalamate, was given, followed

by constant infusion at twelve mL/h. To attain stable plasma concentrations of both tracers, a two hour stabilization period followed, after which the clearance periods start. Clearances were measured over the next two hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer, and P represents the tracer value in plasma at the end of each clearance period. GFR was calculated from UV/P of ^{125}I iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippurate. The day-to-day variability for GFR is 2.5%.

Treatment regimen after transplantation
From January 1988 to February 1993, standard immunosuppression consisted of the following: cyclosporine standard formulation (Sandimmune; Novartis Pharma b.v., Arnhem, The Netherlands; 10 mg/kg; trough levels of 175–200 mg/L for the first three months, 150 mg/L between three and twelve months post-transplant and 100 mg/L thereafter) combined with prednisolone (starting with 20 mg/day, rapidly tapered to 10 mg/day). From March 1993 to May 1997, standard immunosuppression consisted of cyclosporine microemulsion (Neoral; Novartis Pharma b.v., Arnhem, The Netherlands; 10 mg/kg; trough levels idem) and prednisolone. From May 1997 to date, Mycophenolate mofetil (Cellcept; Roche b.v., Woerden, The Netherlands; 2 g/day) was added.

Calculations
Mean arterial pressure (MAP), was calculated as: MAP = (1/3[systolic pressure – diastolic pressure] + diastolic pressure). Donor single kidney GFR was calculated as: (basal GFR / 2).

Data analyses
Analyses were performed using PASW statistics version 18.0 and GraphPad Prism version 5 for Windows. Data are given as mean ± standard deviation or median [IQR]. Independent samples *t* Test, One-Way ANOVA and Mann-Whitney U test were used to analyze differences between groups.

Data on underlying kidney disease, pre-emptive character of the transplantation, ischemia times, HLA mismatches, occurrence of rejection, immunosuppressant and antihypertensive drug use and statin use were collected for all recipients. To evaluate which transplantation related factors were associated with one and five year recipient GFR and ERPF, we performed univariate regression analysis. Factors with a P-value ≤ 0.05 in univariate analysis were entered into a backward linear regression analysis combined with donor age, BMI, GFR and MAP, and were removed in successive steps at a threshold of P-value ≤ 0.05.

Results
Donor and recipient characteristics are shown in tables 1 and 2. Of the 195 recipients who received a kidney more than five years ago, 49 recipients had no five year follow-up due to death of the recipient (n=12), graft failure (n=14), or unknown reasons (n=21). There was no difference in donor and recipient baseline characteristics between pairs available and unavailable for follow-up. Of all 113 recipients, 28% went through a rejection episode and 6 and 3% developed graft failure and died more than five year after transplantation.

Transplantation related factors, evolution after transplantation, drug regimen and use of antihypertensive drugs are listed in table 3, as well as univariate associations between these factors and recipient GFR and ERPF at one and five year post-transplantation.

Table 1: pre-donation donor characteristics and donor risk profile.

	Total
N (% male)	113 (39)
Age at Unx (years)	49 ± 10
Age > 50 years (n, %)	16 (15)
BMI (kg/m^2)	25 ± 4
Obesity (n, %)	11 (10)
MAP (mmHG)	92 ± 9
Hypertension (n, %)	18 (16)
Smoking behavior	
Current smoking (n, %)	37 (33)
Former smoking (n, %)	28 (25)
No smoking (n, %)	48 (42)
GFR (mL/min)	114 ± 19
ERPF (mL/min)	432 ± 80
Risk factors	
0 risk factors (n, %)	30 (26)
1 risk factor (n, %)	62 (55)
2 risk factors (n, %)	17 (15)
3 risk factors (n, %)	4 (4)

Values represent N (%), mean ± SD or median [IQR]. Obesity is defined as BMI>30 kg/m^2, hypertension as donor antihypertensive drug use or a blood pressure > 150/85 mmHg.

Recipient outcome by donor risk factors

Occurrence of donor risk factors is shown in table 1. Although only 10-15% of donors were above 60 years of age, obese or hypertensive, 55% had one risk factor and 15% had two risk factors combined. Recipient kidney function at one and five year post-donation by a break-up in donor risk factors is shown in figure 1. No significant differences were found between recipients of donors with or without the different risk factors, albeit numerical differences were observed for donor age and hypertension. For neither of the time points, a difference in the occurrence of rejection, graft failure and recipient death could be detected between the groups.

Regression analysis

In univariate regression analysis, recipient glomerular filtration rate (GFR) at one and five year post-transplantation positively related to donor pre-donation GFR (R 0.30 and 0.39, both $p<0.01$). Similarly, recipient ERPF at one and five year after transplantation related to donor ERPF (R 0.36 and 0.32, both $p<0.01$). Donor age correlated negatively to recipient GFR and

Table 2: recipient characteristics at one and five year post transplantation.

	One year follow-up	Five year follow-up
Duration of follow-up (years)	1.1 ± 0.2	5.1 ± 0.2
N (% male)	113 (60)	113 (60)
Age (years)	41 ± 13	45 ± 13
BMI (kg/m^2)	26 ± 4	26 ± 5
Systolic BP (mmHg)	140 ± 15	134 ± 17
Diastolic BP(mmHg)	85 ± 11	82 ± 10
MAP (mmHG)	103 ± 11	99 ± 12
GFR (mL/min)	59 ± 15	58 ± 19
ERPF (mL/min)	238 ± 53	226 ± 62
Urinary protein excretion (g/24h)	0.2 [0.0-0.3]	0.2 [0.0-0.4]

Values represent N (%), mean ± SD or median [IQR].

ERPF at five year post-donation (R -0.24 and -0.20, p<0.01), but not to one year kidney function. Donor BMI and MAP did not associate to recipient outcome at one or five years post-transplantation.

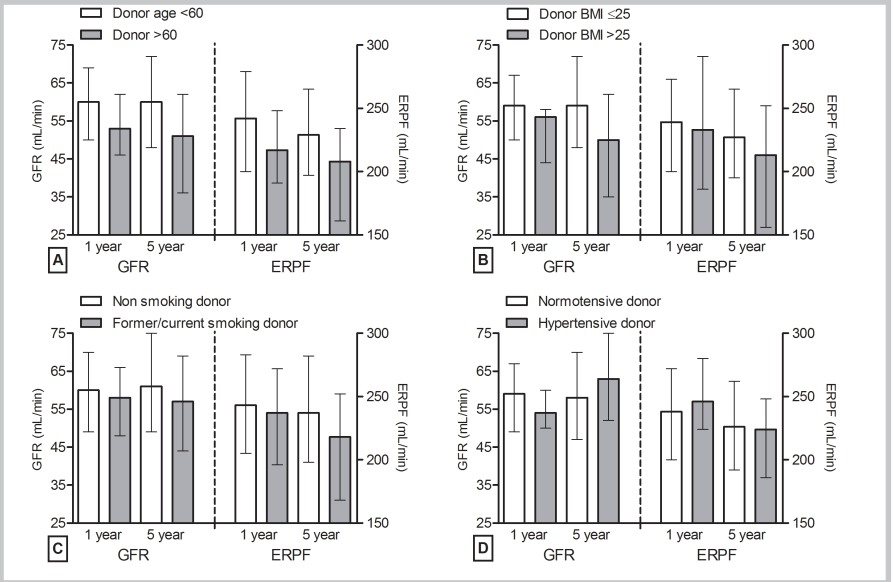

Figure 1: one and five year recipient GFR (left bars) and ERPF (right bars) by a break-up in donor characteristics: a) donor age, b) donor BMI c) donor smoking behavior and d) donor hypertensive status. Bars represent median [IQR]. No differences were found between the groups.

Table 3: Transplantation related characteristics, evolution post-transplantation and drug use in recipients.

		Five year outcome				Five year outcome			
		GFR		*ERPF*		*GFR*		*ERPF*	
	Value	Std. β	*P* value	Std. β	*P* value	Std. β	*P* value	Std. β	*P* value
Primary renal disease (n, %)									
Primary glomerular disease	12 (11)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Glomerulonephritis	32 (28)	-0.04	0.69	-0.01	0.94	-0.06	0.50	-0.01	0.96
Tubular interstitial disease	3 (3)	-0.01	0.93	-0.01	0.92	0.11	0.23	0.01	0.95
Polycystic kidney disease	17 (15)	-0.05	0.62	-0.09	0.37	-0.11	0.23	-0.09	0.34
Dysplasia and hypoplasia	19 (17)	0.12	0.22	0.22	0.02	0.15	0.11	0.14	0.16
Renovascular disease	13 (11)	-0.15	0.12	-0.13	0.19	-0.10	0.32	-0.07	0.47
Diabetes Mellitus	3 (3)	-0.03	0.75	-0.06	0.67	-0.14	0.14	-0.10	0.30
Other or unknown cause	14 (12)	-0.11	0.25	-0.07	0.47	0.03	0.80	-0.01	0.91
Related donor (n, %)	81 (72)	0.06	0.52	-0.04	0.67	0.03	0.77	-0.01	0.91
Pre-emptive procedure (n, %)	34 (30)	-0.06	0.51	-0.12	0.23	-0.09	0.33	-0.09	0.36
Previous transplant (n, %)	8 (7)	-0.03	0.75	-0.10	0.30	0.01	0.96	0.02	0.86
Warm ischemia times (min)	40 ± 18	0.11	0.23	0.08	0.38	0.10	0.32	0.04	0.67
Cold ischemia times (min)	157 ± 83	-0.12	0.19	-0.15	0.11	-0.17	0.08	-0.13	0.18
HLA-AB mismatches (n)	1.8 ± 1.1	-0.02	0.88	-0.02	0.83	-0.03	0.76	-0.06	0.67
HLA-DR mismatches (n)	0.8 ± 0.7	-0.18	0.06	-0.17	0.07	-0.14	0.15	-0.19	0.05
Acute rejection (n, %)	33 (29)	-0.08	0.44	-0.10	0.32	-0.09	0.37	-0.01	0.92
Drug use									
Cyclosporine (n, %)	46 (41)	0.02	0.80	0.09	0.35	-0.06	0.53	0.09	0.36
Trough-level (µg/L)	95 ± 45	-0.03	0.82	0.15	0.18	-0.21	0.15	-0.20	0.18
Tacrolimus (n, %)	18 (16)	-0.03	0.80	-0.09	0.37	0.08	0.39	-0.06	0.50
Trough-level (µg/L)	8.0 ± 3.0	-0.41	0.04	-0.14	0.50	-0.22	0.41	-0.24	0.37
Prednisolone dose (mg/day)	9.0 ± 1.6	-0.02	0.90	0.00	0.99	0.02	0.83	0.03	0.79
Azathioprine (n, %)	17 (15)	-0.13	0.26	-0.17	0.12	-0.12	0.22	-0.09	0.38
Mycophenolate mofetil (n, %)	89 (79)	0.11	0.32	0.15	0.17	0.12	0.23	0.05	0.62
Number of antihypertensives	1.7 ± 1.0	0.06	0.60	0.00	0.98	-0.33	0.01	-0.25	0.01
ACE-inhibitor or AII-antagonist (n, %)	59 (52)	0.25	0.02	0.26	0.02	-0.13	0.19	-0.03	0.76
β-blocker	71 (63)	-0.12	0.27	-0.22	0.05	-0.17	0.09	-0.17	0.10
Calcium channel blocker	23 (20)	0.13	0.24	0.10	0.40	-0.01	0.92	0.03	0.75
Statin	5.1 ± 1.2	-0.0-	0.36	-0.07	0.44	-0.12	0.20	-0.14	0.16
Total cholesterol (mmol/L; %)	52 (46)	-0.03	0.82	-0.15	0.19	-0.01	0.94	-0.15	0.15

Values represent mean ± SD and median [IQR]. Standardized β (Std. β) and P-value were obtained by univariate regression analysis with either recipient GFR or ERPF one or five year post-transplantation as dependent variable. ACEi: angiotensin converting enzyme inhibitor; AII: angiotensin II.

Combined effect of donor characteristics

Univariate associations between transplantation related factors, post-transplantation evolution and drug use and recipient GFR and ERPF are shown in table 3. To evaluate the combined effects of donor factors and transplantation related factors, linear regression was used. For one-year recipient GFR, the strongest model was formed by solely the tacrolimus trough level (std. β -0.42; adjusted R^2 0.14; p<0.01). For recipient ERPF, the model contained only donor ERPF (std. β 0.36; adjusted R^2 0.12; p<0.01). For five-year recipient outcome, GFR was best predicted by donor GFR and the number of antihypertensive drugs (std. β 0.36 and -0.31; adjusted R^2 0.22; p<0.01). Recipient ERPF was predicted by donor ERPF (std. β 0.32; adjusted R^2 0.10; p<0.01). Other donor risk factors and transplantation related factors had no significant influence in the abovementioned models.

Discussion

In this study, we evaluated the influence of living donor renal risk profile, i.e. donor age, BMI, smoking behavior and blood pressure, on recipient renal function. One-year recipient outcome was solely related to donor kidney function and not to other donor characteristics. For five-year recipient outcome, higher donor age associated recipient kidney function as well, though in a negative manner. Overall recipient kidney function was good both in recipients of high and of low risk donors.

This is the first study that evaluates influence of the donor renal risk profile on recipient renal outcome in living kidney donation with detailed renal function measurements in both donor and recipient. Although overall recipient renal function was good, with a GFR of approximately 60 mL/min, we found a negative influence of higher donor age on recipient renal outcome.

Previous studies evaluating the importance of donor factors for recipient outcome focused mainly on donor age. Though higher age is a risk factor for lower GFR in the recipient (3,5,6,25,28), older donors are an important extension of the donor pool (4,23,24,29-31). Here, we reported a negative association between donor age and recipient renal function, at five year post-transplantation. However, quantitatively the effect was modest, amounting to an average 5 mL/min for each additional 10 years of donor age. Accordingly, neither at one nor at five years post-transplantation the difference seen between recipients of older and younger donors reached statistical significance.

Donor overweight and/or obesity is associated with more delayed graft function, more acute rejection and lower renal function in the recipient, although graft and patient survival are similar (26,27). Here, we show no effect of donor BMI on short-term outcome, neither for kidney function nor for rejection or graft failure.

Smoking is recognized as a potential risk factor for the development of CKD (15-18). Smoking of the recipient prior to kidney transplantation increases the risk for graft loss (32) and mortality (19). Autopsy studies suggested that smoking may affect the renal vasculature (14). Such lesions may not be reflected in renal function due to the large compensatory capability of the kidney. Thus, donors who smoke may have mild, non-detectable, kidney damage. Previously, Leunissen found that renal histological lesions amplify the nephrotoxic effects of calcineurin inhibiting drugs (33). Hereby, recipients of smoking donors may bear more nephrotoxic effects with subsequent graft damage. This is in line with a study in lung transplant recipients, which showed a dose dependent association between former smoking and the development of CKD after lung transplantation (34). Here, however, we find no detrimental effect of donor smoking behavior. This may be due to an overall

relative good health of our living donors, who are selected for good renal function.

Issa et al. found that higher donor blood pressure was associated with lower estimated GFR in the recipient (5). In our center, donors with pre-existent hypertension were not accepted for donation before 2002, resulting in only a small number of recipients of hypertensive donors with long term follow-up, which resulted in low power to detect an effect of donor blood pressure on long term recipient renal function. This low power may explain the apparent lack of influence of donor blood pressure on long-term recipient outcome. Alternatively, selection for a true GFR > 80 mL/min may have selected donors whose kidneys are relatively resistant to the effects of higher blood pressure, as suggested by our recent observation that hypertensive donors have a renal outcome that is indistinguishable from that in normotensive donors (35).

Our study has several limitations, the most important being the small sample size of the follow-up cohort, and the mono-centric character, that may limit the generalizabllity of our data. As our patients were predominantly Caucasian, the conclusions do not apply to patients with African or other ethnicity. Furthermore, our follow-up may have been too short to detect any detrimental effects of donor characteristics, since Giral et al. recently showed that detrimental effects may not appear earlier than seven years post-donation (36).

What are the implications of this study? Here, we evaluated the effect of the donor risk profile on recipient renal outcome, i.e. the effect of the use of more marginal living kidney donors. Higher donor age, blood pressure and body mass index were associated with lower recipient renal function. The effects, however, were limited, and overall recipient renal function was sufficient. Thus, in the population presented here, no major detrimental effects of the donor risk profile were seen. However, caution is warranted in the interpretation of these results. First, we would like to emphasize that donors at our centre undergo thorough screening, with the use of gold standard renal function measurements, and the eligibility to donate is based on a true GFR > 80 mL/min, or, in exceptional cases, like subjects older than 70 years of small females, with a GFR > 70 mL/min which responses very well to dopamine infusion (stimulated GFR > 80 mL/min). Thus, accepted donors with one or more risk factors represent a healthy subset of their peers, as also apparent from the small or absent impact of the named risk factors on pre-donation renal function. So, even the older and hypertensive donors presented in this study have good renal function. Mean BMI was 26, which may hamper extrapolation to other populations, for instance United States donors, where the proportion of overtly obese donors is considerable. Here, we choose the cutoffs for donor age and BMI in according to the literature, a donor age of 60 years (3,23-25) and BMI of 30 kg/m^2 (26,27). In our population, however, an age of 50 years and BMI of 25 kg/m^2 creates more equal sized groups. When we performed analyses with the latter cutoffs, we found similar results as with the more severe cutoffs (data not shown). Based on our data, we conclude that accepting living donors with extended criteria is a safe way to enlarge the donor organ pool, without detrimental effects for the recipient, provided that careful screening is performed.

In summary, this study shows modest negative effects of donor age, and no effect of donor BMI, blood pressure or smoking behavior on recipient renal outcome, with a good overall recipient renal function. Therefore, we conclude that the change in donor characteristics has not resulted in worse recipient outcome in the medium term. Studies with more long term follow-up, in a donor population with more extended characteristics, are necessary to ensure long term safety.

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General discussion

Living kidney donation

Since the first successful transplantation in Boston, 1954, kidney transplantation has evolved to the preferred treatment of end stage kidney failure, with superior quality of life and survival compared to dialysis (1-5). While deceased donor organs have been the main source for transplantation for many years, living kidney donors have become increasingly important in many countries and constitute now half of the kidney transplants performed in the Netherlands (6). As outcome after living donation is substantially better than with deceased donor kidneys, the living donation program has become of major importance in renal replacement therapy. Against the background of the persistent deceased donor shortage many centers aim to maximize the living donor pool, without compromising donor safety. Although living kidney donation is a safe procedure, the fact that the donor is left with a single kidney renders him or her more vulnerable to injury or functional loss of the remnant kidney. Therefore, in general, potential donors are obliged to have excellent kidney function, and also be in excellent health to be accepted for kidney donation. Considering the rigorous selection criteria, it is not surprising that studies evaluating long term donor outcome all report good outcome long term and show no increased risk for the development of kidney damage compared to the general population. With growing experience and a growing request for donor organs, selection criteria for potential donors have become more liberal (7;8). Although the required renal functional limits remained unchanged, with a lower clearance limit of 80 mL/min for kidney donation, many centers have now extended their upper limit for age accepting donors beyond 65 years. In fact, even donors over 70 years are considered for recipients older than 60 years in special programs. For BMI, the upper limit of 30 kg/m^2 was adjusted according to national prevalence of obesity, which is rising worldwide. Since a few years, many centers started to accept donors with a past medical history of regulated hypertension as well. The changes in the selection of the donor population may have consequences for donor as well as recipient outcome and it becomes more and more important to monitor the living donation program for such consequences. In this thesis we investigate the impact of the changes in risk profile of the donor population on donor and recipient outcome at the University Medical Center Groningen.

Kidney function

Kidney function assessment is one of the cornerstones of donor evaluation. To allow living donation for both donor and recipient, kidney function prior to donation has to be sufficient to ensure adequate renal function with one kidney. In the past the cutoff was set at a clearance of 40-60 mL/min, but due to a publication by Bia et al. in 1995 consensus was reached to use the threshold of 80 mL/min (9). Nowadays, about 75% of centers still use 80 mL/min as the lower limit for kidney donation. The remaining 25% requires kidney function to be within two standard deviations of the mean kidney function for the donor's age, or even have a cutoff of 90 mL/min (8). Donors in our center are selected with a lower threshold of 80 mL/min. To date no functional kidney impairment after donation has been documented. Of note is that the level of kidney function prior to donation mainly predicts post-donation kidney function, both for donor and recipient.

Gold standard assessment of kidney function using radioactive isotopes or iodinated

tracers is expensive, and is therefore only applied by 10% of all centers evaluating potential donors. The majority relies on estimates of kidney function by 24 h creatinine clearance or creatinine-based kidney function equations. Both methods, however, have distinct shortcomings. Whereas creatinine-clearance and creatinine-based equation have a large non-systematic error, the equations, moreover, systematically underestimate kidney function, especially when the latter is in the normal range, as it is with potential kidney donors. Collection of 24 h urine has the benefit that other renal parameters such as proteinuria, can be evaluated as well. For most centers, proteinuria measurement is part of the standardized donor screening. Cutoffs for proteinuria vary between 150 and 300 mg/day (8).

Donor age

Increasing age is associated with a decline in kidney function (10-14). This raises the question about the suitability of older individuals for kidney donation, from a donor perspective as well as for recipient outcome. With ageing, the number of functioning nephrons declines and changes in the tubuli, glomeruli and vascular bed occur (15-23) that can affect the compensatory response to nephron loss. In ***chapter five*** and ***nine***, we found a lower GFR prior to donation in older donors, with age being an independent determinant of GFR. In line with this lower pre-donation GFR, in older donors early post-donation GFR is lower as well. Fortunately, the long-term compensatory response to nephrectomy in older donors was similar to younger donors with a parallel increase in GFR from early post-donation up to 5 years after donation for donors older or younger than 55 years. Other studies reported good post-donation adaption as well (24-27) supporting the safety of donation in terms of kidney function for older donors.

Donor age is a main consideration from a recipient perspective as well. Since older donors have lower kidney function, less kidney function is transplanted with the graft of an older donor. Accordingly, higher donor age results in lower recipient kidney function (28-33). In line, we found that donor age was the most important donor factor next to donor GFR in predicting recipient GFR (***chapter 9***). Although kidney function of recipients of older donors is lower, it is sufficient, and there was no increased rate of function decline or incidence of graft loss in our study. Thus, older donors can be an important extension of the donor pool. Further studies are, however, necessary to evaluate the effects on long-term.

Influence of gender and body size

Influence of donor gender on donor and recipient outcome was debated in several studies. In the past the majority of living donors in the University Medical Center Groningen was female, while gender distribution is more equal nowadays. Although gender has never been a selection criterion, some believe that female kidneys perform worse after transplantation, especially when transplanted into a male recipient, as shown in deceased donors (34;35). Women are generally smaller than men, and, hence, have less nephron mass with a lower kidney function compared to men. Rather than a structural property of the kidney, this lower kidney function simply reflects lower body dimensions, and, consequently, a lower metabolic demand in women. This is not likely to affect donor outcome, but might be relevant to kidney function and outcome in the recipient, where the metabolic demands might be different from

those of the donor. We find a lower kidney function in female donors than in male donors. When GFR was normalized for BSA, however, the difference between male and female donors disappeared suggesting that the difference is due to differences in body dimension rather than to intrinsic renal differences.

After donation, male donors may be at a higher risk of loss of kidney function than female donors in the very long term (36;37). This risk, however, is similar to the risk in the general population, where men are at higher risk as well (38-41).

Several studies have shown a negative influence of female donor gender on recipient outcome (34;35;42;43), others demonstrated a definite influence of recipient gender (44;45). Gender differences were explained by higher metabolic demands in male recipients, and by the graft/recipient weight ratio (42;44;46-48). As female kidneys are smaller (49), they may not be able to live up to the high metabolic demands of male recipients. Body size disparity between donor and recipient may lead to lower graft function due to smaller kidney dimensions. This may explain the difference in outcome between female and male kidneys after post-mortal transplantation. Whether this also applies to a healthy donor kidney has been subject of discussion (45;48;50-52). In ***chapter eight*** we found no influence of donor or recipient gender on recipient outcome up to five year after transplantation. The kidney appears to adapt to body size of the recipient, irrespective of donor or recipient gender. In fact, kidneys of smaller donors show a larger increase in kidney function after transplantation than kidneys of larger donors, demonstrating that the transplanted kidney adapts to the metabolic demands of its new environment, both on short and long term. This is associated however, with a slightly higher urinary protein excretion early after donation. This could be a subtle sign of hyperfiltration and a maladaptive response, but fortunately, it disappears in the long term follow-up, suggesting that in our population the adaption of a small kidney to a large recipient's metabolic demand did not have harmful consequences long-term. We must emphasize,however, that our donor population is thoroughly screened, has an excellent kidney function before donation and the generalizability of our findings to settings where donor screening is less strict is uncertain.

Donor overweight and obesity

Overweight and obesity is a growing problem worldwide and it also affects potential donors rendering them to a possibly increased risk for kidney damage. As in the general Dutch population, the body mass index of the donor pool has risen during the last decade. Although a BMI < 30 kg/m^2 is preferable for surgery, not all centers have the luxury to be able to reject potential donors when their BMI exceeds 30 kg/m^2. The risk of excess weight, however, already starts at values of BMI in the overweight range. Already in the overweight range BMI is an independent determinant of an unfavorable renal hemodynamic profile, already in the overweight range, and several studies in the general population have shown a link between overweight and long-term risk for end stage kidney failure (38;53-58). In donors, higher BMI was associated with lower kidney function and lower reserve capacity post-donation (11;59). Furthermore, obesity was reported as a risk factor for post-donation kidney function impairment (60;61). Others, however, found no influence of BMI on post-donation outcome (62-64). In ***chapter five*** we saw no influence of BMI on donor outcome five years after living

donation. Post-donation kidney function, as well as the adaption to nephrectomy was similar between normal and overweight donors, and no signs of hyperfiltration were found.

While overweight and obesity in the recipient are clearly associated with the risk of graft failure (65;66), the effects of donor BMI on recipient outcome are less clear. Although recipients of very obese donors (BMI > 35 kg/m^2) show more delayed graft function and acute rejection episodes (67;68), there were no differences in occurrence of graft failure or in recipient kidney function. In ***chapter nine*** we found no effect of donor BMI on recipient outcome, neither for kidney function, nor for the occurrence of rejection or graft failure. There were, however, no very obese donors operated in our population, which somewhat limits the generalizability and comparison of our data to other populations, with more prominent obesity, such as in the US.

Donor blood pressure and hypertension

Hypertension is one of the best well known risk factors for the development of kidney damage in the general population (69-73). The acceptance of hypertensive individuals for kidney donation, therefore, has long been a subject of debate. While in the past it was absolutely unthinkable to accept a donor with pre-existent hypertension, since 2002, the University Medical Center Groningen accepts potential donors who use antihypertensive drugs, provided blood pressure is well controlled. Whether these donors are at increased risk for kidney function loss remains unknown. Many studies reported an increase in blood pressure in previously normotensive donors post-donation (59;74-78), although not invariably so (79). Donors who developed hypertension after donation had higher macro albuminuria and urinary protein excretion in three studies (78;80;81), and were at higher risk for a low kidney function (82). A follow-up study of pre-existent hypertensive donors, however, found similar outcome in hypertensive and normotensive donors (83). However, only three of these donors had hypertension that warranted antihypertensive treatment, so the relevance of these data to the current donor population is uncertain.

In ***chapter four***, we compare outcome of 49 donors using antihypertensive drugs prior to donation, to matched, normotensive, controls. We found no detrimental effects of pre-existent hypertension on short term and five year post-donation outcome. Remarkably, control donors had an increase in blood pressure post-donation, but the hypertensive donors maintained stable blood pressure. Moreover, post-donation course of kidney function was similar in hypertensive and normotensive donors and none of the donors developed proteinuria (***chapter 5***). This favorable outcome in hypertensive donors might come as a surprise, considering the role of hypertension as a renal risk factor. Whereas these results are encouraging, we must emphasize that our donors were selected for a GFR over 80 mL/min. In particular in hypertensive individuals this may have selected those in whom the kidney is not particularly susceptible to renal damage.

In kidney transplantation, hypertensive traits of the donor may be transferred to the recipient, as shown in animal studies and human transplantation. Several studies have demonstrated that higher donor blood pressure is associated with lower kidney function in the recipient (30;84-87). In ***chapter nine***, we found a similar negative correlation between donor blood pressure and recipient kidney function one year after transplantation.

Furthermore, donor hypertension increased the risk of a GFR < 60 mL/min in the recipient. Nevertheless, outcome of recipients from a hypertensive donor was good. As noted above, the hypertensive donors were selected for good kidney function, and may have represented a specific selection of the hypertensive population. Under these conditions, donation of hypertensive donors was safe for donor and recipient.

Donor smoking behavior

The effects of smoking on donor outcome are largely unknown. Next to the known effects of smoking on the development of cardiovascular disease and malignancies, smoking is recognized as a potential risk factor for the development of chronic kidney disease (58;88-93). Smoking can affect the kidney vasculature, and such lesions may make a kidney more prone to nephrotoxicity of calcineurin inhibitors (94). Thus, recipients of smoking donors may bear more nephrotoxic effects with subsequent graft damage. In line, recipient smoking increases the risk for graft loss and mortality (95;96), and in lung transplant recipients a dose dependent association between former smoking and the development of CKD after lung transplantation was found, demonstrating a long-lasting effect of prior smoking (97). Smoking, however, is not a selection criterion for potential kidney donors. Fortunately, in ***chapter nine*** we found no harmful effects of smoking prior to donation on post-donation kidney function course, and, moreover, we find no effect of the donors' smoking behavior on recipient outcome, neither on short, nor on long term post-transplantation.

Implications for donor screening

To justify living donor programs, long-term donor safety needs to be ensured. This emphasizes the need of thorough screening of potential donors. As mentioned above, we found no effects of pre-donation BMI, hypertension or smoking behavior on post-donation outcome in donors or recipients. These favorable findings should be interpreted in the context of the thorough screening for overall health, and particularly, kidney function. Pre-donation kidney function is the main predictor of post-donation kidney function in donor and recipient. Accurate kidney function measurements are therefore of major importance for donor screening. Since true kidney function measurement is expensive, and requires specific expertise, many centers rely on an estimation of kidney function (eGFR) for donor screening by use of kidney function equations. The two most widely used equations, the Modification of Diet in Renal Disease (MDRD) Study equation and Cockcroft-Gault (CG) equation (98;99), however, were derived from populations with kidney function impairment, and perform poorly in individuals with normal kidney function (100-107). More recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was developed in a large data set in different populations including subjects without renal disease (108), to aim for better performance in subjects without overt renal disease. In ***chapter one***, we found that the CKD-EPI equation was indeed more accurate and precise than the MDRD Study and CG equation in estimating pre-donation kidney function. Nevertheless, kidney function was significantly underestimated by all three equations. Thus, use of these equations will lead to rejection of many potential donors that actually have adequate kidney function. If a cut-off of an eGFR <80 mL/min/1.73m^2 would have been used in the 253 donors presented in ***chapter one***, 55, 31 an 25% of donors would

have been rejected by use of MDRD, CKD-EPI and CG, while all had sufficient pre- and post-donation kidney function! Thus, the use of kidney function equations many lead to unwarranted and substantial reduction of the donor pool, putting unnecessary pressure on the waiting lists for kidney transplantation. Therefore, we plead for thorough donor screening with preferably gold standard kidney function measurement, or, alternatively, repeated creatinine clearances.

Although kidney donors are selected because of good health and kidney function, minor morphological damage, like glomerulosclerosis, interstitial fibrosis and tubular atrophy, may be present at time of donation (18;19;109-112). Due to the large reserve capacity of the kidney, such changes may not be reflected in lower kidney function. Consequences for post-donation donor outcome are unclear. Associations have been described between mild baseline damage and graft performance in the recipient (112-121). Thus, kidney biopsies may be an important tool to assess the risks for the donor and recipient. ***Chapter seven*** evaluates whether morphological, pro-fibrotic and pro-inflammatory changes in baseline kidney biopsies associate to donor characteristics and early post-donation outcome. The pro-fibrotic marker α-smooth muscle actin (α-SMA) associated to donor age and kidney function prior to and early post-donation. Older donors had higher α-SMA expression, while donors with a BMI > 30 kg/m^2 had a higher number of interstitial macrophages and more vascular intima-thickening than leaner donors. None of the baseline changes, however, associated with the change in kidney functio over donation. Thus, although baseline damage is present these changes appear to be too mild to affect early post-donation renal adaptive capacity. Effects on long term donor and recipient outcome, however, need to be evaluated by future studies.

Implications for donor follow-up

Although thorough screening should assure long-term donor safety, accurate donor follow-up remains of major importance. With increasing age, donors may develop hypertension, glucose intolerance or micro-albuminuria, all undesirable in the single kidney situation, but may be treated well when early detected. Furthermore, any decrease in kidney function should be detected early, so that early intervention can be started. As described above, many centers use kidney function equations to monitor kidney function. These equations perform poorly in subjects with normal kidney function such as kidney donors. After donation, when kidney function is lower, performance is somewhat better (***chapter one***). To be feasible for donor follow-up, however, the kidney function equations should be reliable to detect kidney function loss over time. Somewhat surprisingly, validation studies on kidney function equations so far almost exclusively focused on cross-sectional data. Therefore, we evaluated the use of kidney function equations for monitoring of kidney function over time, in CKD patients and former kidney donors respectively. In both populations we found that mean performance of the equations is acceptable (***chapter two*** and ***three***). However, progressive kidney function decline is not detected reliably, which is a major drawback for its clinical use in the clinic. Therefore, for donor follow-up, kidney function equations should be interpreted with caution. Preferably, gold standard methods, or serial creatinine clearances should be used. Furthermore, other markers for kidney damage should be evaluated during donor follow-up, for instance 24 h urinary protein excretion, to assure early detection of kidney

damage.
Many former kidney donors have an estimated or measured GFR below 60 mL/min/1.73m^2, the upper limit of CKD stage 3 kidney disease (122). Former kidney donors with a kidney function below this threshold, therefore, may be marked as CKD patient, with possible negative socio-economic consequences. The current CKD staging, however, is based on the presence of two kidneys. A low GFR in a subject with two kidneys may reflect kidney disease, while the same GFR in a former donor reflects the presence of a single, healthy, kidney, as supported by the excellent long term donor outcome. The prognostic impact of a given level of GFR is one of the main factors underlying the current CKD staging system. In ***chapter six***, therefore, we compare the kidney function course of former donors to that of matched CKD patients to evaluate the impact of CKD staging for prediction of donor outcome. As expected, CKD patients show progression of their CKD stage over time. In former donors, by contrast the CKD stage improves from early post-donation to long term. Thus, the CKD staging has no predictive value in donors, And former donors with a GFR <60 mL/min/1.73m^2 should not be regarded as CKD patients. Yet, one should be aware of their reduced kidney function in conditions where drug toxicity is related to reduced kidney function.

In summary, the population of living donors has evolved over the last decades, with older, more overweight and even hypertensive donors. In this thesis we have evaluated the effects of these factors on living donor and recipient outcome in the donor population of our center. Fortunately, we found no detrimental effects of donor age, BMI, blood pressure or smoking behaviour on donor and recipient outcome. It is important, however, to mention that our donors are thoroughly screened, including gold standard kidney function measurements. Thus, even donors with an adverse risk profile are ensured of good kidney function prior to donation, which is probably a main factor in the encouraging outcome in donor and recipient. This emphasizes the importance thorough screening of potential donors. Thus, when pre-donation kidney function is good, acceptation of older, overweight and hypertensive donors is safe for donor and recipient. Although post-donation outcome of donors presented here was good, we would like to plead for structural, long term donor follow-up, including overall health assessment as well as a reliable follow-up of kidney function. Taken together, this will allow to optimize the donor pool, to the benefit of patients, without compromising donor safety.

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Nederlandse samenvatting

Inleiding

Wereldwijd stijgt het aantal patiënten met een nierziekte. Niertransplantatie is de voorkeursbehandeling van eind stadium nierfalen. Gezonde mensen kunnen veilig een nier afstaan voor transplantatie. Doordat er een groot tekort is aan donornieren van overleden donoren, zijn levende nierdonoren steeds belangrijker geworden voor niertransplantatie. In Nederland wordt de helft van alle niertransplantaties verricht met behulp van een levende donor. Nieren van levende donoren zijn kwalitatief beter dan nieren van overleden donoren, wat voor de ontvanger van de nier veel voordelen biedt.

Gezonde nieren hebben veel reservecapaciteit. Wanneer één nier verwijderd wordt kan de andere nier dit compenseren door harder te gaan werken. Lange termijn studies naar donor en ontvanger uitkomst laten dan ook goede resultaten zien. Om aan de groeiende vraag naar donornieren te voldoen, zijn de criteria voor potentiële donoren de afgelopen tien jaar echter versoepeld. Voorheen kwamen alleen zeer fitte, volledig gezonde mensen in aanmerking voor nierdonatie. De afgelopen jaren is de leeftijdsgrens voor potentiële donoren verdwenen, worden donoren met overgewicht en zelfs obesitas geaccepteerd, en sinds 2002 worden ook donoren die bloeddrukverlagende medicatie gebruiken geaccepteerd. Hiertegenover staat wel dat de nierfunctie van een potentiële donor voldoende hoog moet zijn. Het is bekend dat de nierfunctie daalt met de leeftijd. Overgewicht, obesitas en een hoge bloeddruk zijn bekende risicofactoren voor het ontwikkelen van nierschade op de lange termijn. De verschuiving in de donorkarakteristieken, naar oudere donoren die vaker overgewicht of een hoge bloeddruk hebben, zet dus vraagtekens bij de lange termijn uitkomst van de donor én de ontvanger van de nier. In dit proefschrift onderzoeken we daarom de effecten van de nieuwe donor karakteristieken op de nierfunctie van donor en ontvanger uitkomst vijf jaar na de transplantatie. Daarnaast proberen we een antwoord te geven op hoe we de levende donor het beste kunnen volgen na donatie.

Om antwoord te geven op onze vragen hebben we de nierfunctie gemeten van de levende donoren en hun ontvangers. In de donoren hebben we de nierfunctie vier maand voor donatie en twee maand en ongeveer vijf jaar na donatie gemeten. In de ontvanger is de nierfunctie drie maanden, één jaar en vijf jaar na transplantatie gemeten.

Deel I: geschatte nierfunctie voor screening en follow-up

In de dagelijkse praktijk wordt de nierfunctie op verschillende manieren gemeten. De meest nauwkeurige 'gouden standaard' methode maakt gebruik van radioactieve stoffen. Dit is een tijdrovende en kostbare manier van nierfunctie meten. Een andere methode is het meten van de 'creatinineklaring'. Hiervoor moet echter 24 uur urine verzameld worden, wat helaas vaak tot meetfouten leidt. Daarom wordt veelvuldig gebruik gemaakt van formules die de nierfunctie kunnen schatten met behulp van één bloedwaarde. Deze formules zijn goedkoop en gemakkelijk in het gebruik. Uit eerdere studies is echter gebleken dat de schatting van de nierfunctie minder precies wordt naarmate de nierfunctie hoger is, met een onderschatting van de echte nierfunctie. In het eerste deel van dit proefschrift laten we zien dat in levende donoren de schatting van de nierfunctie voor donatie – bij een hoge nierfunctie op twee gezonde nieren – meer afwijkt van de gouden standaard waarde dan na donatie, wanneer de nierfunctie lager is. Het presteren van de formules is dus afhankelijk van de hoogte van de

echte nierfunctie. Tevens laten we zien dat wanneer de formules gebruikt worden voor het screenen van potentiële donoren, bijna de helft van de donoren zou worden afgewezen omdat de nierfunctie te laag wordt ingeschat terwijl de echte nierfunctie voldoende is.

Omdat de nierfunctie na donatie lager is dan voor donatie – er is immers een nier verwijderd – geven de formules na donatie een redelijk goede schatting van de nierfunctie. Voor het volgen van de nierfunctie na donatie zouden de formules dus een goedkoop en gebruiksvriendelijk alternatief kunnen zijn. We hebben zowel in nierpatiënten als in donoren na donatie onderzocht hoe goed de formules het nierfunctieverloop over langere termijn kunnen inschatten. Zowel in de nierpatiënten als in de nierdonoren schatten de formules het nierfunctieverloop goed in. Wanneer de nierfunctie echter snel daalt wordt dit niet opgepikt door de formules. Dit zet vraagtekens bij de toepasbaarheid van de formules voor het vervolgen van de nierfunctie van donoren.

Deel II: effecten van het donor risico profiel op uitkomst van de donor

In dit tweede deel van het proefschrift onderzoeken we de effecten van de donor karakteristieken voor donatie op de korte en lange termijn uitkomst van de donor na donatie. Allereerst hebben we een groep donoren die voor donatie bloeddrukverlagende medicatie gebruikten vergeleken met een groep controle donoren die voor donatie dergelijke medicatie niet gebruikten. Omdat donoren met bloeddrukverlagende medicatie in ons centrum pas sinds 2002 worden geaccepteerd voor donatie, is de lange termijn uitkomst nog zeer beperkt. Op korte termijn, twee maand en één jaar na donatie, zien we geen verschillen in de nierfunctie van donoren met of zonder bloeddrukverlagende medicatie. Ook zien we dat bij de donoren met medicatie de bloeddruk niet méér stijgt dan bij de controle donoren en dat er niet meer medicijnen nodig zijn na donatie.

Vervolgens hebben we de invloed van het pre-donatie risicoprofiel op de lange termijn uitkomst van de donor onderzocht. Risicofactoren waren een leeftijd boven 55 jaar, overgewicht en obesitas, hoge bloeddruk en roken. Donoren ouder dan 55 hadden zoals verwacht voor en na donatie een lagere nierfunctie dan jongere donoren. De daling in nierfunctie over de donatie en het herstel van de nierfunctie naar de langere termijn was echter gelijk tussen oude en jongere donoren. Overgewicht, hoge bloeddruk en roken lieten geen negatieve effecten op de donor uitkomst zien.

Na donatie is de nierfunctie van een deel van de donoren dusdanig verlaagd dat deze donoren volgens de meest gebruikelijke classificatie voor nierziekten, die berust op bepaalde afkappunten van nierfunctie, een nierziekte zouden hebben. Deze classificatie gaat er echter van uit dat de nierfunctie verlaagd is bij twee zieke nieren, terwijl donoren na donatie een lagere nierfunctie hebben met één gezonde nier. We hebben daarom het nierfunctie verloop van donoren van twee maand na donatie tot vijf jaar na donatie vergeleken met dat in nierpatiënten. Waar de donoren echter na vijf jaar follow-up een toename in nierfunctie laten zien, daalt in de nierpatiënten de nierfunctie over de tijd. Hoewel donoren na donatie een lage(re) nierfunctie hebben, is dit dus geen teken van nierziekte.

Hogere leeftijd, overgewicht en hoge bloeddruk zijn risicofactoren voor het ontwikkelen van nierschade. Ook in gezonde nieren kan lichte schade aanwezig zijn, zonder dat dit de nierfunctie beïnvloedt. Tijdens de donatieprocedure worden er weefsel biopten uit de nier

genomen. In deze biopten blijkt ook in gezonde donoren zeer milde schade aanwezig. Bij oudere donoren is er meer schade aanwezig in de nier dan bij jongere donoren, op grond van het normale verouderingsproces. We zagen geen invloed van overgewicht of hoge bloeddruk. Hoewel meer schade samenging met een lichte verlaging in de nierfunctie, werd het aanpassingsvermogen van de nier, en dus de uitkomst van nierfunctie na donatie, niet beïnvloed door de aanwezigheid van die schade.

Deel III: effecten van het donor risico profiel op de uitkomst van de ontvanger
In het derde deel onderzoeken we de effecten van het donor profiel op de uitkomst van de ontvanger van de nier. Er zijn veel discussies over de invloed van het geslacht en de lichaamsgrootte van de donor en ontvanger op de uitkomst van de transplantatie: zo wordt wel beweerd dat de kwaliteit van vrouwennieren slechter is dan van mannennieren. Daarom hebben wij naar het effect van de geslachts- en lichaamsgrootteverhouding tussen de donor en ontvanger op de nierfunctie van de ontvanger vijf jaar na transplantatie gekeken. Uit onze studie blijkt dat de nierfunctie zich aanpast aan de lichaamsgrootte van de ontvanger: een nier van een kleine donor gaat harder werken in een grote ontvanger dan in een kleine ontvanger. Het geslacht van de donor en de ontvanger lijkt hierin geen rol te hebben.

In het tweede deel hebben we gezien dat donor leeftijd, overgewicht, bloeddruk en rookgedrag geen invloed hebben op de aanpassing van de nier na donatie. In het laatste hoofdstuk verbreden we ons onderzoek naar de effecten op de uitkomst van de ontvanger vijf jaar na transplantatie. Hoewel we wel een negatief effect vonden van hogere leeftijd en hogere bloeddruk van de donor op de nierfunctie van de ontvanger, hebben de ontvangers van oudere donoren en donoren met een hoge bloeddruk wel een goede – zij het iets lagere – nierfunctie vijf jaar na transplantatie. Er was geen invloed op de overleving van de ontvanger. Donor overgewicht en rookgedrag hadden geen invloed op de overleving en nierfunctie van de ontvanger.

Conclusies en implicaties
Samenvattend vinden we in dit proefschrift geen nadelige effecten van de nieuwe donorkarakteristieken – oudere leeftijd, overgewicht en hoge bloeddruk – op de uitkomst van de donor en de ontvanger vijf jaar na de donatie of transplantatie. De nierfunctie van de donor vóór donatie blijkt de belangrijkste voorspeller van de uitkomst van de donor en ontvanger. Deze resultaten werden verkregen in een cohort donoren waarin zorgvuldige screening plaatsvond, met een ondergrens voor pre-donatie nierfunctie van 80 mL/min. Mogelijk speelt deze selectie een rol bij de gunstige uitkomsten. Mits de donor goed gescreend is voor donatie lijken de huidige selectiecriteria dus veilig op de middellange termijn. Verdere studies zijn echter nodig om de effecten op de lange termijn vast te stellen. Daarom blijft het belangrijk om donoren na donatie goed te volgen om bij eventueel nierfunctie verlies vroeg te kunnen ingrijpen. Hier ligt ook potentieel een belangrijke rol voor leefstijlinterventie, hoewel het onderzoek naar de effecten hiervan nog in volle gang is. Voor het volgen van donor nierfunctie na donatie zijn nierfunctieformules een goedkoop en gebruiksvriendelijk alternatief voor gouden standaard metingen. Hierbij moeten de tekortkomingen van deze formules echter wel in acht genomen worden.

Nederlandse samenvatting

Dankwoord

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